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(54) Title: METHOD AND APPARATUS FOR PLASMA DEPOSITION OF CHEMICALLY REACTIVE GROUPS ON SUBSTRATES CHEMICALLY REACTIVE SUBSTRATES OBTAINABLE BY THE METHOD AND USE THEREOF
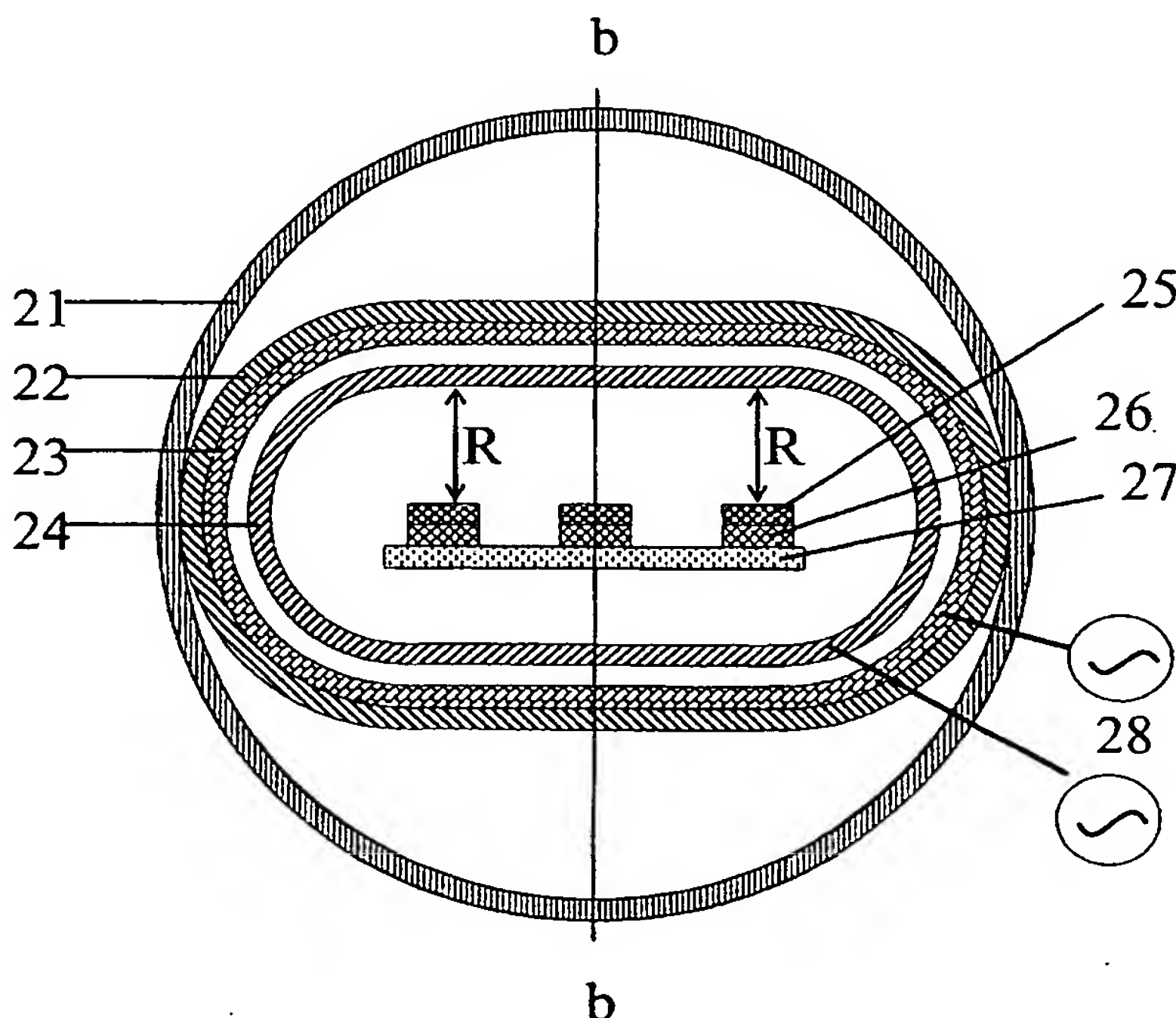
(57) Abstract: The present invention relates to a method and apparatus for plasma deposition of a chemically reactive group (Y-Z) on a substrate, chemically reactive substrates, and use thereof, e.g. for immobilisation of biomolecules; the method comprising: (a) providing at least one precursor (A-X (Y)) for the chemically reactive group; (b) providing at least one donor (D(Z)), said at least one donor comprising at least one addition group (Z), optionally said at least one addition group (Z) being comprised in said precursor (A-X (Y)) and optionally said at least one donor (D(Z)) is not being provided; (c) providing a substrate (M); (d) providing a gas plasma, said gas plasma having a pressure and an energy to form at least one activated carrier group (B); and (e) reacting said substrate (M), said at least one precursor (A-X (Y)), said at least one donor (D(Z)) in said gas plasma so that said chemically reactive group (Y-Z) is bound to said substrate, either directly (M-Y-Z) or via said at least one activated carrier group (M-B-Y-Z), and so that when exposed to a substance which chemically reacts with said chemically reactive group, said substance binds thereto.

METHOD AND APPARATUS FOR PLASMA DEPOSITION OF CHEMICALLY
REACTIVE GROUPS ON SUBSTRATES, CHEMICALLY REACTIVE SUB-
STRATES OBTAINABLE BY THE METHOD, AND USE THEREOF

5

DESCRIPTION

1. BACKGROUND OF THE INVENTION

10   The present invention relates to a method and apparatus
for plasma deposition of a chemically reactive group on a
substrate, chemically reactive substrates, and use there-
of, e.g. for immobilisation of biomolecules.

15   The Technical Field

Generally, immobilisation of biomolecules on substrates
is achieved by reacting functional groups of the bio-
molecules to chemically reactive groups of the substrate.

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Chemically reactive groups of the substrate comprise
functional groups of the substrate material itself and
functional groups of substances attached or bonded to the
substrate material by suitable bonding. Bonding includes
25   e.g. covalent, ionic, or affinity bonding either directly
to the substrate or indirectly to the substrate through
spacers and intermediate layers of coatings such as base
coating and intermediate coating.

30   Generally, functional groups of the substrate are located
at the surface of the substrate material, however, the
nature of the surface may include inner surfaces of the
substrate material, e.g. for folded or porous surfaces.

Chemically reactive groups of the substrate comprises chemical groups such as amine $-NH_2$, thiol $-SH$, hydroxyl $-OH$, epoxy $-HC-O-CH-$, aldehyde $-CHO$, carboxylic acid $-COOH$, acidic anhydride $-CH_2CO-O-COCH_2-$, and carbonyl
5   chloride $-COCl$, which are fixed to the substrate material either directly or indirectly through spacers and intermediate compounds.

Bonding of these chemically reactive groups to the sub-
10   strate involves use of substances which are very reactive and may cause damages of process equipment, e.g. damages in form of corrosion of surfaces by acidic or basic decomposition reactions by acids and acidic chlorides and by basic amines.
15

Further, these reactive substances are often hazardous to humans and the environment, and may cause serious odour complaints, especially reactive substances such as thiols and acidic chlorides.
20

Consequently, there is a need for a method of providing chemically reactive groups on a substrate whereby these prior art disadvantages are avoided.

25   Furthermore there is a need for a method which enables fabrication of surfaces with a higher density of chemically reactive groups than can be obtained with the use of said above mentioned reactive substances alone, e.g. if said hazardous effects are dealt with.
30

Generally, a prior art plasma deposition apparatus comprise two or more electrodes connected to a power supply, both of alternating current or direct current types, said

electrodes being adapted to generate a plasma by electrical glow discharge in a gas introduced between them.

In such plasma, however, the gas is subjected to plasma
5   of high energy, providing a high degree of decomposition of the excited gas. Further, such high energetic plasma is often inhomogeneously distributed, resulting in an inhomogeneous distribution of the excited gas.

10 Consequently, there is a need for a plasma deposition apparatus which avoids a high degree of decomposition of excited gas and for which the distribution of excited gas can be better controlled.

15 Prior Art Disclosures

US 4 633 809 discloses an amorphous silicon film-forming apparatus comprising a base and a facing electrode forming a plasma generating region there between and being
20 surrounded by a grounded mesh structure for preventing gas radicals from dispersing outside the mesh structure.

EP 0 741 404 B1 discloses a method and an electrode system for excitation of a plasma.
25
WO 00/44207 discloses a method of excitation of plasma by means of a plurality of electrode systems.

2. DISCLOSURE OF THE INVENTION
30
Object of the Invention

It is an object of the present invention to seek to provide a method and apparatus for preparing a substrate

with chemically reactive groups, and use of such sub-strates.

In an aspect, it is the object of the present invention to seek to provide a method involving less corrosive chemicals for the synthesis of the chemically reactive groups

It is a further object of the present invention to seek to provide a method involving less toxic chemicals for the synthesis of the chemically reactive groups

It is a still further object of the present invention to seek to provide a method involving less environmentally harmful chemicals for the synthesis of the chemically reactive groups.

It is a still further object of the present invention to seek to provide a method providing a high density of chemically reactive groups.

In another aspect, it is the object of the present invention to seek to provide an improved apparatus for carrying out the method.

It is a further object of the present invention to seek to provide an apparatus for carrying out the method which apparatus can be used to prepare large numbers of sub-strates with chemically reactive groups in one batch.

It is a further object of the present invention to seek to provide an apparatus for carrying out the method which apparatus can be used to prepare substrates with chemi-

cally reactive groups with little variation in quality between prepared substrates in one batch.

It is a further object of the present invention to seek to provide an apparatus for carrying out the method which apparatus can be used to prepare substrates with chemically reactive groups with little variation in quality between prepared substrates from different batches.

Further objects appear from the description elsewhere.

Solution According to the Invention

"Method of plasma deposition of chemically reactive groups on a substrate"

According to the present invention, these objects are fulfilled by providing a method of plasma deposition of a chemically reactive group Y-Z on a substrate M, the method comprising:

(a) providing at least one precursor for the chemically reactive group, said precursor A-X(Y) comprising:

at least one carrier group A, and

at least one cleaving group X(Y), said at least one cleaving group comprising at least one active group Y, said at least one active group comprising one or more atoms, said at least one active group having at least one bond, at least one of said at least one bond being cleavable, and said at least one active atom being bonded to said at least one carrier group;

(b) providing at least one donor D(Z), said at least one donor comprising at least one addition group Z, said at least one addition group having at least one bond, said at least one bond of said at least one addition group being cleavable, said at least one addition group being able to bond to said at least one active group,

optionally said at least one addition group Z being comprised in said precursor A-X(Y) and optionally said at least one donor D(Z) not being provided;

(c) providing a substrate M;

(d) providing a gas plasma, said gas plasma having a pressure and an energy to cleave said at least one cleavable bond of said at least one cleaving group, to cleave said at least one cleavable bond of said at least one addition group, and to form at least one bond between said at least one active group of said at least one cleaving group and said at least one addition group, and forming at least one activated carrier group B; and

(e) reacting said substrate M, said at least one precursor A-X(Y), said at least one donor D(Z) in said gas plasma;

wherein the extent of reaction of said substrate, said at least one precursor, said at least one addition group, said pressure of said gas plasma, and said energy of said gas plasma is selected so that said chemically reactive group Y-Z is bound to said substrate, either directly M-Y-Z or via said at least one activated carrier group M-B-Y-Z, and so that when exposed to a substance

which chemically reacts with said chemically reactive group, said substance binds thereto.

It has surprisingly turned out that a chemically reactive group can be deposited on a substrate by reaction of a chemically less reactive precursor molecule with a donor molecule in plasma. This requires that the precursor contains a cleaving group which can be cleaved in the plasma and that at the same plasma conditions also the donor can be cleaved thereby liberating an addition group, which can then bond to the cleaved cleaving group of the precursor under the formation of the chemically reactive group.

Furthermore, it has turned out that when the precursor contains certain carrier groups which can be activated in the plasma the chemically reactive group can be effectively bound to the substrate through the activated carrier group.

Finally, the chemically reactive groups thus deposited are able to effectively bind a complementary chemical substance to the substrate, i.e. the surface properties of a substrate can be improved in terms of the ability to bond to it specific chemical substances.

Preferred embodiments are defined in the subclaims and the description elsewhere.

<u>Preferred embodiments</u>

In a preferred embodiment said precursor A-X(Y) comprises a leaving group L, said leaving group bonding to said cleaving group A-X(Y)-L whereby said leaving group can be

made to leave said cleaving group in said gas plasma
thereby making a less reactive precursor molecule more
reactive for the formation of the chemically reactive
group Y-Z.

5

In another preferred embodiment, said precursor, pre-
ferably the carrier group A, carries groups comprising
said addition group Z whereby said carrier group and said
addition group can be supplied with the same gas stream
10   and the number of gasses to be fed to the plasma can be
reduced.

In a preferred embodiment, precursors form primary amine
$-NH_2$ on a substrate. Such precursors are selected from
15   secondary amines, tertiary amines and nitriles. The addi-
tion group Z in all cases being hydrogen H. The hydrogen
can be supplied as part of the precursor, or it can be
supplied as part of a non-corrosive donor gas, e.g. H_2,
methane CH_4, or it can be supplied as part of the precur-
20   sor and at the same time be supplied as part of a donor
gas. Alternatively ammonia NH_3 can be used as donor gas.
The disadvantage of using NH_3 as donor gas is the fact
that it is corrosive and hazardous, and the advantage of
using NH_3 as donor gas is that a very high surface den-
25   sity of amine can be achieved.

"Chemically reactive group Y-Z: $-NH_2$ from secondary amine
precursors"

30   In a preferred embodiment, said precursors forming pri-
mary amine $-NH_2$ on a substrate from secondary amines are
selected from the group consisting of: saturated secon-
dary amines, unsaturated secondary amines, saturated he-
terocyclic secondary amines, saturated substituted hete-

rocyclic secondary amines, unsaturated heterocyclic sec-
ondary amines, unsaturated substituted heterocyclic sec-
ondary amines, and combinations thereof.

5   In a particularly preferred embodiment, said secondary
amines are selected from the group consisting of: sat-
urated secondary amines: di-ethylamine; unsaturated sec-
ondary amines: di-allylamine and N-allylmethylamine; sat-
urated heterocyclic secondary amines:pyrrolidine and
10 piperidine; saturated substituted heterocyclic secondary
amines; unsaturated heterocyclic secondary amines: pyr-
role and imidazole; and unsaturated substituted hetero-
cyclic secondary amines: 2,4-di-methylpyrrole, and com-
binations thereof.
15
The advantage of using secondary amines as precursors in
a feed gas of the plasma deposition method according to
the present invention is that secondary amines generally
present a lower risk to the health and to the environment
20 (a substantial amount of the molecules fed to the plasma
chamber exit the vacuum pumps unaltered) and generally,
that secondary amines are less corrosive than the corre-
sponding primary amines.

25 <u>"Chemically reactive group Y-Z: $-NH_2$ from tertiary
amines"</u>

In a preferred embodiment, said precursors forming pri-
mary amine $-NH_2$ on a substrate from tertiary amines are
30 selected from the group consisting of: saturated tertiary
amines, unsaturated tertiary amines, saturated hetero-
cyclic tertiary amines, saturated substituted hetero-
cyclic tertiary amines, unsaturated heterocyclic tertiary

amines, unsaturated substituted heterocyclic tertiary amines, and combinations thereof.

In a preferred embodiment, said precursors forming pri-
mary amine $-NH_2$ on a substrate from tertiary amines are selected from the group consisting of:

saturated tertiary amines: tri-ethylamine,
unsaturated tertiary amines: tri-allylamine,
saturated substituted heterocyclic tertiary amines: 1-
methylpyrrolidine,
unsaturated heterocyclic tertiary amines: pyridine and pyrimidine, and
unsaturated substituted heterocyclic tertiary amines: 1-
methylpyrrole, and
combinations thereof.

The advantage of using tertiary amines as feed gas rather than primary amines is that tertiary amines generally
present a lower risk to the health and to the environment and generally are less corrosive than the corresponding primary amines.

"Chemical reactive group Y-Z: $-NH_2$ from nitriles"

In a preferred embodiment, precursors forming primary amine $-NH_2$ on a substrate are selected from the group consisting of: nitriles, saturated nitriles, unsaturated nitriles, and aromatic nitriles.

In a preferred embodiment, said nitriles are selected from the group consisting of: acetonitrile, n-butane-nitrile, acrylonitrile, and benzonitrile.

The advantage of using nitriles as feed gas rather than primary amines is that nitriles generally are less corrosive than e.g. the corresponding primary amines.

5   "Chemically reactive group Y-Z: -thiol (-SH) from precursors of thiolethers"

In a preferred embodiment, the addition group is hydrogen H. The hydrogen can be supplied as part of the precursor,
10   or it can be supplied as part of a non-corrosive donor gas, e.g. H_2, methane CH_4, or it can be supplied as part of the precursor and at the same time be supplied as part of a donor gas. Alternatively hydrogen sulphide, H_2S, can be used as donor gas. The disadvantage of using H_2S as
15   donor gas is the fact that it is hazardous, and the advantage of using H_2S as donor gas is that a very high surface density of thiol can be achieved.

Formation of thiol (-SH) from precursors selected from
20   thiolethers, such as saturated thiolethers, e.g. tert-butylsulfide), unsaturated thiolethers, e.g. allylsulfide and allylmethylsulfide, saturatet heterocyclic thiolethers, e.g., tetrahydrothiophene, unsaturated heterocyclic thiolethers, e.g. thiophene, and unsaturated sub-
25   stituted heterocyclic thiolethers, e.g. 2-methylthiophene and 2,5-di-methylthiophene, and combinations thereof.

The advantage of using thiolethers as feed gas rather than thiols is that thiolethers generally present a lower
30   risk to the health and generally are less irritating (stench) than the corresponding thiols.

When forming amines and thiols it is preferred that no oxygen is present in the feed gas. As an example it is

known from prior art, see e.g. WO 00/20656, that cyano-
acrylate can be effectively bonded to a surface by a
plasma process. Furthermore, cyanoacrylate contains a
nitrile group from which a primary amine can be formed in
5   accordance with the present invention. However, cyano-
acrylate also contains two oxygen atoms which can be
activated in plasma and which can interfere with the
desired reactions leading to the formation of primary
amine. Therefore cyanoacrylate is less preferred choice
10   of precursor for the formation of primary amine by the
method outlined in the present invention.

"Chemically reactive group Y-Z: -carboxylic acid -COOH
from precursors comprising esters, aldehydes, and keto-
15   nes"

In a preferred embodiment, precursors forming carboxylic
acid on a substrate are selected from esters, such as
saturated esters, e.g. ethyl acetate, ethyl isobutyrate,
20   methyl isobutyrate; unsaturated esters e.g. methylmeth-
acrylate, ethyl propiolate, methyl propiolate; cyclic
esters, e.g. beta-butyrolactone, gamma-valerolactone), or
a combination thereof.

25   For all these precursors, the addition group is hydrogen
H. The hydrogen can be supplied as part of the precursor
or it can be supplied as part of a donor gas, e.g. water
H_2O, H_2, methane CH_4, or it can be supplied as part of the
precursor and at the same time be supplied as part of a
30   donor gas.

Formation of carboxylic acid from precursors selected
from aldehydes, addition group being hydroxyl OH. The
hydroxyl can be supplied as part of the precursor or it

can be supplied as part of a donor gas, e.g. water H_2O, methanol CH_3OH, or it can be supplied as part of the precursor and at the same time be supplied as part of a donor gas.

Formation of carboxylic acid from precursors selected from ketones, addition group being hydroxyl OH. The hydroxyl can be supplied as part of the precursor or it can be supplied as part of a donor gas, e.g. water H_2O, methanol CH_3OH, or it can be supplied as part of the precursor and at the same time be supplied as part of a donor gas.

The advantage of using esters, aldehydes, or ketones as feed gas rather than carboxylic acids is that esters, aldehydes, and ketones generally present a lower risk to the health and to the environment and generally are less irritating and corrosive than the corresponding carboxylic acid.

"Chemically reactive group Y-Z: -carbonyl chloride -COCl from precursors comprising esters, aldehydes, and ketones"

In a preferred embodiment, the addition group Z is chloride Cl. The chloride can be supplied as part of the precursor, or it can be supplied as part of a non-corrosive donor gas, e.g. tri-chlorofluoromethane $CFCl_3$, di-chlorofluoromethane $CHFCl_2$, ehtylchloride C_2H_5Cl, di-chloroethane $C_2H_4Cl_2$, chloropropane C_2H_7Cl, di-chloropropane $C_2H_6Cl_2$, or it can be supplied as part of the precursor and at the same time be supplied as part of a donor gas. Alternatively chlorine, Cl_2, can be used as

donor gas. The disadvantage of using Cl_2 as donor gas is the fact that it is hazardous, and the advantage of using Cl_2 as donor gas is that a very high surface density of carbonyl chloride can be achieved.

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In a preferred embodiment, precursors forming carbonyl chloride are selected from esters, such as saturated esters, e.g. ethyl acetate, ethyl isobutyrate, methyl isobutyrate; unsaturated esters, e.g. methylmethacrylate,

10   ethyl propiolate, methyl propiolate; cyclic esters, e.g., beta-butyrolactone, gamma-valerolactone. In these cases chlorine is provided by the donor gas.

In a preferred embodiment, precursors forming carbonyl

15   chloride are selected from aldehydes.

In a preferred embodiment, precursors forming carbonyl chloride are selected from ketones.

20   "Chemically reactive group Y-Z: -carbonyl chloride -COCl from precursors comprising chlorinated esters, chlorinated aldehydes, and chlorinated ketones"

In a preferred embodiment, precursors forming carbonyl

25   chloride are selected from chlorinated esters, chlorinated aldehydes, and chlorinated ketones.

The advantage of using esters, aldehydes, or ketones as feed gas rather than carbonyl chlorides is that esters,

30   aldehydes, and ketones generally present a lower risk to the health and to the environment and generally are less irritating and corrosive than the corresponding carbonyl chlorides.

"Donor D(Z)"

In preferred embodiments, the donor supplies the addition group Z for the chemically reactive group. However, optionally, the precursor comprises the addition group Z.

In the gas plasma process, the donor is cleaved, or optionally the precursor is cleaved, and liberates the addition group. Preferred donors are in form of donor gas, including gasses such as H_2, H_2O, CH_4, and NH_3 and chlorine containing substances such as tri-chlorofluoromethane $CFCl_3$, di-chlorofluoromethane $CHFCl_2$, ethylchloride C_2H_5Cl, di-chloroethane $C_2H_4Cl_2$, chloropropane C_2H_7Cl, di-chloropropane $C_2H_6Cl_2$ whereby reduction by H_2 or CH_4 (less environmentally hazardous than H_2) by reducing N-C to N-H or S-C to S-H, hydroxylation of C-C to C-OH, amine formation of C-C to C-NH, and chlorination of C=O to C-Cl or C-C to C-Cl can be obtained.

"Substrate with chemically reactive groups"

In another aspect the present invention provides a substrate with a chemically reactive group obtainable by the method according to the invention whereby substrates with new chemically reactive groups can be obtained, e.g. chemically reactive groups which otherwise would be decomposed by the plasma. Also, substrates with chemically reactive groups having no or having reduced amounts of hazardous chemical substances residues can be provided.

"Use of substrates with chemically reactive groups"

In another aspect, the present invention provides use of such a substrate in a chemical reaction between a chemi-

cally reactive substance and the chemically reactive group on the substrate whereby various new separation techniques such as scavenging using fibres with chemically reactive groups, and assay techniques, including im-
5   mobilization, can be obtained.

In a preferred use, said chemically reactive substance is immobilised to the chemically reactive group on the substrate whereby substances such nucleic acids, e.g. DNA,
10   and proteins can immobilized.

In another preferred use, said immobilised chemically reactive substance is a sensor for chemically reactive compounds, preferably biological compounds, and/or envi-
15   ronmental compounds whereby compounds such as e.g. anti-bodies, receptors, DNA, and proteins can be measured, e.g. by a cantilever-based biosensor or by a quartz-crystal micro-balance based sensor these sensors having such chemically reactive groups deposited on the can-
20   tilever surface and on one of the electrodes of the micro-balance, respective.

In still another preferred use, said immobilised chemi-cally reactive substance is a DNA sensor.
·25
In still another preferred use, said immobilised chemi-cally reactive substance is used in a method for screen-ing of DNA, or in an apparatus for screening of DNA.

30   "Apparatus for plasma deposition of chemically reactive groups on a substrate"

In another aspect, according to the present invention, these objects are fulfilled by providing an apparatus for

plasma deposition of a chemically reactive group on a substrate, the apparatus comprising:

(a) a vacuum pump for providing a vacuum;

5

(b) a vacuum section comprising:

(i) a vacuum outlet connected to said vacuum pump,

10   (ii) one or more gas inlets for supplying one or more gasses therein, and

(iii) one or more gas outlets for discharging residual gasses therefrom;

15

(c) a plasma gas section, said plasma gas section being arranged in said vacuum section for accommodating a gas plasma, and said plasma gas section comprising two or more electrodes arranged to generate a gas plasma there-

20 in, said vacuum section and said plasma gas section being electrically insulated;

(d) a reaction section, said reaction section being arranged in said plasma section and being adapted to

25 receive at least a part of said gas of plasma, and said reaction section comprising a substrate holder adapted to receive a substrate and to expose said received substrate to said received gas of plasma; and

30 (e) a power supply, said power supply being adapted to provide one or more phases of one or more alternating voltages for providing a plasma between said two or more electrodes, said alternating voltage being supplied in

one or more phases equal to or less than the number of said two or more electrodes.

Preferred embodiments are defined in the subclaims and description elsewhere.

In a preferred embodiment, one or more each of said one ore more phases comprises one or more conventional lamps, said lamps being connected in series with said one or more electrodes to stabilise the current, I, of each phase.

At low values of I the resistance R of a lamp is low and constant with regard to I. At intermediate and high values of I, R increases with increasing I. In the case that I increases rapidly, e.g. due to the formation of an electrical arch between neighbouring electrodes, R will increase and thereby decrease I. This in turn will eliminate said electrical arc. Thus the non-linear current-resistance characteristic of conventional lamps can be used to effectively stabilise the plasma density in AC powered plasmas.

The power supply should be able to supply a sufficient electrode voltage for the plasma to ignite and should be able to supply a sufficient power for the desired reactions to take place within the plasma.

"The plasma power density"

The reactions going on in the plasma strongly depend on the average plasma power density, ρ_{plasma} which is conveniently defined as the ratio of the electrical power over the total plasma gas volume, V_{plasma},

$$\rho_{plasma} = \text{electrical power}/V_{plasma} \qquad (1)$$

where the electrical power is given by the product of the measured voltage over the electrodes multiplied by the measured current of the power supply output, and where the gas plasma volume is given by the volume of the plasma section. Depending on the electrode set-up it can be difficult to determine the exact boundary of the plasma volume and thereby the total plasma volume. As a rule of thumb the gas inside the plasma volume emits visible light, thus the position of the plasma volume boundaries can be estimated by observing the plasma from various angles. However, this is not always practically achievable. In the case of AC diffusion plasma, the total plasma volume to a good approximation is given by the volume encompassed by the outermost electrodes. E.g., for a cylindrical electrode set-up of radius R and length H, the total plasma volume, V_{plasma}, is given by

$$V_{plasma} = H \times \pi \times R^2 \qquad (2)$$

In the following this definition of total plasma volume is used to calculate average plasma densities in accordance with equation (1).

The average plasma power density, ρ_{plasma}, can be accurately controlled by varying the following plasma process parameters: Electrode voltage, pressure, and gas composition. Within a certain process window ρ_{plasma} increases with increasing electrode voltage and increases with increasing pressure. The dependence of ρ_{plasma} on the gas composition is quite complex. However, the presence of easily ionised and otherwise inert gasses such as the noble gasses en-

sures the existence of plasma even at low average power densities such as preferred in the present invention. For typical feed gas compositions the process window is defined by electrode AC voltages in the range 200-2000 V and pressures in the range 0.01-1 mbar.

For a number of popular electrode geometries approximate values of V_{plasma} can be calculated in accordance to equation (1) by inserting proper values of H and R; e.g., for an inductively coupled plasma with a cylindrical coil wound around a cylindrical vacuum container, H is given by the length of the coil and R is given by the inner radius of vacuum container. For plasma sustained between two parallel circular electrodes, H is the distance between the electrodes, and R is the radius of the electrodes.

It should be noted that values for the plasma density for any given plasma section, which geometrical form may deviate from that of the cylindrical electrode set-up in equation (2), can be calibrated against such a standard plasma section. This can be achieved by optical emission spectroscopy (OES) measurements of the emitted light from the plasma, since the intensity of the emitted light is quite sensitive to the power density. For the purpose of calibration pure noble gas plasma is preferred. For any such noble gas a large number of emission wave lengths are present in the emitted light. However for each noble gas, the intensity of certain wavelengths are dominating and therefore especially useful for performing such a calibration, e.g., in the case of a pure argon plasma the intensity of the optical emission at 434.8 nm is relatively intense, and for a pure helium plasma the emission at 587.6 nm is strong.

For a continuous dc or ac source, the plasma power density can be expressed as the average power consumption of the plasma divided by the volume over which the plasma

5   exists. For a pulsed electrical source the plasma power density can be expressed as the average power consumption of the plasma during the pulse divided by the volume over which the plasma exists.

10   In a preferred embodiment, said at least one power supply supplying said two or more electrodes and said gas section are adapted to provide an average plasma power density in the range including 0.01 to 100 W/l, preferably 0.1 to 10 W/l, more preferably 0.1 to 5 W/l, most

15   preferably 0.1 W/l to 3 W/l, and in particular about 1 W/l, whereby suitably "soft" energies can be used. By the term "soft" energies is meant sufficiently low energy to avoid production of excessive amounts of undesired decomposition products of the precursor or of other gasses

20   present; such "soft" energies still being high enough to cause the desired cleaving of the precursor.

<u>"Vacuum pump"</u>

25   The vacuum pump for providing a vacuum can be any suitable vacuum pump which is able to provide a vacuum of sufficient low pressure to ensure that steady homogeneous plasma can be sustained in the reaction section.

30   Typical vacuum pressures are in the range 0.005 mbar to 10 mbar, preferably 0.010 mbar to 1 mbar, most preferred 0.010 mbar to 0.200 mbar whereby it is obtained that steady and homogeneous plasma is achieved.

Specific vacuum pumps comprise diffusion pumps, rotary vane vacuum pumps, rotary piston vacuum pumps, roots vacuum pumps. Cascading vacuum pumps can be applied.

5   On the high-pressure side, the vacuum pump is connected to a suitable exhaust gas flow system, including mechanical booster pumps, rotary pumps etc.

It is preferred that the pressure can be controlled in a
10 control loop comprising a pressure gauge, an electronic controller device, e.g., a PID controller, and an actuator connected to said electronic controller device. Said actuator may comprise a reduction valve, e.g., a butterfly valve, inserted between the vacuum pump and the vac-
15 uum chamber, or said actuator may comprise a variable vacuum pump, e.g., a roots vacuum pump.

A high vacuum chamber volume to total feed gas flow ratio ensures that minor variations in gas flow does not affect
20 the homogeneity of the plasma.

The vacuum section is coupled to the vacuum pump in any suitable manner known to a skilled person, including flexible steel pipes and rigid steel pipes.

25

"Vacuum section"

The vacuum section is the volume inside the vacuum chamber in which there is a vacuum. Typical vacuum pres-
30 sures are in the range 0.005 mbar to 10 mbar, preferably 0.010 mbar to 1 mbar, most preferred 0.010 mbar to 0.200 mbar whereby it is obtained that steady and homogeneous plasma is achieved.

"Plasma gas section"

The plasma gas section is the sub-volume of the vacuum
section in which there is plasma, characterised by the
5   emission of light.

"Power supply"

The power supply should be able to supply a sufficient
10   electrode voltage for the plasma to ignite. Furthermore,
it should be possible to accurately control the electrode
voltage. For AC power supplies this can be achieved by
connecting the electrodes of the plasma apparatus to the
high voltage side of step-up transformers, connecting the
15   low voltage side of said step-up transformers to variable
transformers, said variable transformers being connected
to a common AC power source, e.g. the European standard-
ised 50 Hz, 220 V power grid or the US standardised 60
Hz, 110 V power grid.
20
For each said one ore more phases preferably one or more
conventional lamps are connected in series with said one
or more electrodes to stabilise the current, I, of each
phase. At low values of I the resistance, R, of a lamp is
25   low and constant with regard to I. At intermediate and
high values of I, R increases with increasing I. In the
case that I increases rapidly, e.g. due to the formation
of an electrical arch between neighbouring electrodes, R
will increase and thereby decrease I. This in turn will
30   eliminate said electrical arc. Thus the non-linear cur-
rent-resistance characteristic of conventional lamps can
be used to effectively stabilise the plasma density in AC
powered plasmas.

"Definitions"

"Chemically reactive group Y-Z"

The method of plasma deposition of a chemically reactive
group comprises formation and deposition onto a substrate
of a chemically reactive group for controlled binding of
a substance to the substrate. The chemically reactive
group is bound, preferably firmly bound, to the substrate
and is able to react and bind to a chemical compound with
proper chemical functionality for reacting with the che-
mically reactive group under the formation of a strong
bond between the chemically reactive group and the sub-
stance. In this way the substance will be strongly bound
to the substrate in a controlled way, whereby it is ob-
tained that the substance remains immobilized under ty-
pical test conditions known to a person skilled in the
art of detecting compounds.

The term "strongly bound to" is intended to mean a bond-
ing sufficiently effective for binding the substance to
the substrate. Measure of the strength of a bond is known
in the art, e.g. by immobilisation experiments under con-
trolled conditions. Further, a skilled person would know
how to vary the bonding strength by empirical experiments
with various precursors providing preselected chemically
reactive groups. Examples of strong bonds are covalent
bonds such as C-C, C-O, etc.

"Precursor A-X(Y)"

The chemically reactive group is formed in the reaction
between a precursor and a donor and in the same process
binds to the substrate. Preferably the chemically reac-
tive group binds to the substrate through an activated
part of the precursor. Preferred precursors are e.g.

secondary amines, tertiary amines, and nitriles forming primary amines. Further preferred embodiments are defined elsewhere in the description.

"Carrier group A"

The carrier group is the part of the precursor, which binds to the substrate in cases where the chemically reactive group is not bound directly to the substrate. Preferably the carrier group is a polymerisable group.

In preferred embodiments, the carrier group carries groups comprising said addition group. Preferred carrier groups are e.g. vinyl, acrylate, benzene, alkenes, alkanes and substituted vinyl, acrylate, benzene, alkenes, alkanes.

"Cleaving group X(Y)"

The cleaving group is the part of the precursor which reacts with the donor under the formation of the chemically reactive group. Preferred cleaving groups are e.g. secondary amine, tertiary amine, nitrile, aldehyde, ketone, ester, ether, and sulfide.

"Active group Y"

The active group is a part of the cleaving group which is directly bound to the carrier group. The active group becomes part of the chemically reactive group. Preferred active groups are e.g. nitrogen N, oxygen O, sulphur S, or carbonyl group C=O.

"Donor D(Z)"

Optionally the addition group Z can be comprised in the precursor; or, the donor comprises the addition group Z. In the gas plasma process the donor is cleaved and

liberates the addition group. Preferred donors are in form of donor gas, including gasses such as H_2, H_2O, CH_4, and NH_3 and chlorine-containing compounds such as tri-chlorofluoromethane $CFCl_3$, di-chlorofluoromethane $CHFCl_2$,

5   ethylchloride C_2H_5Cl, di-chloroethane $C_2H_4Cl_2$, chloropro-pane C_2H_7Cl, di-chloropropane $C_2H_6Cl_2$.

"Addition group Z"

In the process the addition group adds to the reactive

10   group under the formation of the chemically reactive group. Preferred addition groups include e.g. hydrogen H, hydroxyl OH, or chloride Cl. The addition group can be provided by the precursor or by the donor gas.

15   "Substrate M"

A substrate is any suitable substrate able to bind to the carrier group. Suitable substrates are stable under vacuum. A substrate can have any suitable shape or form

20   including but not limited to a plate, a rod, a tube, a fibre, a sphere, a flake, a chip, a wafer, and a mem-brane.

25   3. BRIEF DESCRIPTION OF THE DRAWINGS

In the following, by way of examples only, the invention is further disclosed with detailed description of pre-ferred embodiments. Reference is made to the drawings in

30   which

Fig. 1 shows a sketch of the principle of plasma deposi-tion of chemically reactive groups on substrates accord-ing to the invention;

Fig. 2a shows a cross sectional view of a preferred embodiment of the plasma deposition apparatus according to the invention;

5

Fig. 2b shows a longitudinal sectional view along line B-B in Fig. 2a.

Fig. 3a shows a cross sectional view of an embodiment of
10   the plasma deposition apparatus.

Fig. 3b shows a longitudinal sectional view of an embodiment of the plasma deposition apparatus;

15   Fig. 4 shows a cross sectional view of an embodiment of the plasma deposition apparatus comprising two plasma deposition channels;

Fig. 5 shows a cross sectional view of an embodiment of
20   an electrode arrangement;

Fig. 6 shows a functional diagram of a preferred embodiment of plasma deposition apparatus with auxiliary equipment.

25

Fig. 7 shows radioactive images after binding of oligo-SGP1 and SGP3 to plasma polymerised acrylonitrile on PE slides; and

30   Fig. 8 show radioactive images after hybridisation with oligo-SGP4.

4. DETAILED DESCRIPTION

Fig. 1 shows a sketch of the principle of plasma deposi-
tion of chemically reactive groups on substrates accord-
5   ing to the invention.

Plasma deposition is carried out in an apparatus compris-
ing: a vacuum section 11, plasma section 12, reaction
section 13, electrodes 14, substrates 15 wherein the
10   electrodes 14 provides a plasma in the plasma section 12.
This plasma "diffuses" into the reaction section wherein
the plasma, a gas of reactants (inlets and outlets are
not shown), and the substrate reacts, typically on ex-
posed inner and outer surfaces of the substrate.
15
Figs. 2a shows a cross sectional view of preferred em-
bodiment of plasma deposition apparatus according to the
invention. The apparatus comprises a vacuum chamber 21,
an electrical insulation 22, outer electrode 23, inner
20   electrode 24, substrate 25, substrate support 26, and a
substrate holder 27. The electrodes are connected to
power supplies 28.

Fig. 2b shows a longitudinal sectional view along line B-
25   B in Fig. 2a. The apparatus comprises a vacuum chamber
21, an electrical insulation 22, here e.g. a polyester
film, an outer electrode 23, an inner electrode 24, a
substrate 25, a substrate support 26, a substrate holder
27, a feed gas pipe 28, and a vacuum exit 29.
30
Fig. 3a shows a cross sectional view of an embodiment of
the plasma deposition apparatus. The apparatus comprises:
a vacuum chamber 31, an electrical insulation 32, here
e.g. glass cylinders 32a and 32b, an outer electrode 33,

an intermediate electrode 34, an inner electrode 35, a
substrate 36, a substrate support 37, and a substrate
holder 38. The electrodes are connected to power supplies
39.

Fig. 3b shows a longitudinal sectional view of an em-
bodiment of the plasma apparatus. The apparatus compri-
ses: vacuum chamber 31, an electrical insulation 32, here
e.g. glass cylinders 32a and 32b, and polyester film 32c,
an outer electrode 33, an intermediate electrode 34, an
inner electrode 33, a substrate 36, a substrate support
37, a substrate holder 38, a feed gas pipe 39, and vacuum
exit 40.

Fig. 4 shows a cross sectional view of an embodiment of
the plasma deposition apparatus comprising two plasma
deposition channels. The apparatus comprises: an outer
electrode 41, an inner electrodes 42, and substrate
holders 43 whereby a particular high throughput can be
obtained. The electrodes are connected to power supplies
44.

Fig. 5 shows a cross sectional view of an embodiment of
an electrode arrangement. The electrode arrangement com-
prises an outer electrode 51, an inner electrode 51, and
a substrate 53, said electrode being adapted to the shape
of the surface of the substrate whereby a particular
effective and homogeneous plasma deposition can be ob-
tained.

Fig. 6 shows a functional diagram of a preferred embo-
diment of plasma deposition apparatus with auxiliary
equipment. The apparatus comprises: pressurised gas

flasks 601-602, monomer flasks 603-604, flow controllers 605-608, magnetic valves 609-612, vacuum chamber 613, outer electrode 614, inner electrode 615, quartz crystal micro balance 616 for measuring thickness, D, of the
5   deposited film on-line. A pressure gauge 617, pressure set-point 618, butterfly reduction valve 619, roots vacuum pump 620, rotary vane vacuum pump 621, variable electrical transformers 622-623, step-up electrical transformers (1:4) 624-625, electrical lamps (150W each)
10 626-629, ampere meter 630, volt meter (power = U x I) 631.

"Substrate pre-treatment"

15 Substrates may be applied as provided if proper cleaning of the surface has been ensured. If not a proper cleaning treatment might be required. Proper cleaning treatments for a large number of substrates are known in the art. Otherwise a skilled person can easily adapt a proper
20 cleaning procedure for his particular substrate.

"A procedure for glass slides"

A rinsing solution was prepared by mixing one volume of
25 33% hydrogen peroxide with four volumes of 96% sulphuric acid. Submerge the slides in the hot rinsing solution for ten minutes. Briefly move the slides up and down ten times during this step to ensure effective rinsing. Wash the slides by dipping ten times in clean water. Repeat
30 the washing thrice using clean glass equipment and clean water every time. Dry the slides at 60 °C.

5. EXAMPLES

Preferred embodiments of the invention are further il-
lustrated by the following examples.

5

EXAMPLE 1 "Primary amine plasma deposition"

Substrates 25, here 34 microscope glass slides pre-treat-
ed according to the substrate pre-treatment procedure de-
10   scribed above, of length 7.62 cm (3 inch), width 2.53 cm
(1 inch), and thickness 1 mm were placed in a substrate
holder of an embodiment of the plasma deposition appara-
tus according to the present invention. The apparatus
comprised a 300 litres cylindrical plasma chamber equip-
15   ped with a two-phase electrode system (135 litres) as de-
scribed above.

In this embodiment, substrates were treated on one side
only by covering the other sides with a cover substrate
20   26; here a microscope glass slides similar to the de-
position substrate. Each substrate was placed on a sup-
porting slide of same dimensions before being placed in
the chamber.

25   The electrode geometry is illustrated in Fig. 2a and
Fig. 2b, showing a front view and a side view, respecti-
vely.

The electrode geometry comprises two concentric elec-
30   trodes 23,24, an outer electrode 23, and an inner elec-
trode 24 circumferenced by the outer electrode 23.

In this embodiment, the outer electrode 23 is tightly
wrapped in a polyester film 22 to keep plasma from form-

ing on the outside of the outer electrode 23. It is preferred that this polyester film 22 extends the full length of the plasma chamber thereby shielding the inside of the chamber walls from the plasma gas phase.

5

The outer electrode 23 consists of a stainless steel plate of thickness 0.5 mm formed as an ellipsoidal tube with an approximately elliptical cross section, width of 500 mm, height of 240 mm, and length of 1000 mm.

10

The inner electrode 24 consists of a stainless steel grid of thickness 1 mm formed as an ellipsoidal tube with an approximately elliptical cross section, width of 360 mm, height of 100 mm, and length of 1000 mm.

15

The electrodes were connected to a 50 Hz AC power supply with a manually tuneable voltage.

The 34 pairs of supports and substrates were evenly dis-
20   tributed on a substrate holder, here a tray 27, comprising a stainless steel grid electrically isolated from the electrodes 23,24, and placed at the symmetry plane of the inner electrode 24.

25   The inner electrode 24 is preferably designed so those surface elements to be treated receive plasma of similar conditions; e.g. plasma of similar energy density whereby a homogeneous and even distribution of deposited chemical groups is achieved.

30

In an embodiment, this is achieved for substrates of suitable cross-sectional geometries, e.g. rectangular, by selecting a constant distance, R, between each surface element and the inner electrode. Here, the distance R

from the substrate tray 27 to the inner electrode 24 is constant about 50 mm along the width of the product tray 27. If the electrode set-up is designed such that R is too large for some surface elements, e.g. at 20 cm from

5   the inner electrode, the plasma intensity near the substrates will be decreased leading to lower quality of the products obtained.

If R varies too much, e.g. +/-25% for different substrate
10   positions this may lead to variations in the product quality between individual substrates from the same batch.

Furthermore, it is preferred that the substrate holder, here tray, does not extend the full length of the
15   electrodes because the plasma density may become inhomogeneous with respect to the longitudinal position towards the ends of the electrodes.

In this example, the distance from the substrate tray to
20   the end of the electrode set, L, is preferably larger than 40 cm.

A rectangular NaCl crystal was placed on a supporting slide in the middle of the chamber. The feed gas mixture
25   is fed through a tube 28 exiting at the distance L from the product tray. If L becomes too small, e.g. less than 200 mm variations in product quality may occur as a result of variations in monomer gas concentration with respect to substrate position.
30
The glass slides, and the calibration crystal, were subjected to three consecutive gas treatments providing a pretreated surface, a base coating, and a top coating, respectively:

1) <u>Ar-plasma pretreatment</u>: exposure to an Ar-plasma at pressure 0.013 mbar, said plasma being provided by an argon flow of 25 sccm, an AC voltage 1000 V of 50 cycles per second, an electrical power of 500 W, corresponding to a plasma power density of 3.7 W/l, and with a duration of 60 s,

2) <u>Ar/H$_2$-plasma pretreatment</u>: exposure to an Ar-plasma at pressure 0.013 mbar, said plasma being provided by an argon flow of 17 sccm, H$_2$-flow of 7 sccm, an AC voltage 1000 V of 50 cycles per second, an electrical power of 530 W, corresponding to an average plasma power density of 3.9 W/l, and with a duration of 60 s,

3) <u>hydrocarbon base coating</u>: exposure to an hexene/Ar-plasma at a pressure of 0.013 mbar, said base coating plasma being provided by an argon flow of 25 sccm, and an AC voltage 800 V of 50 cycles per second, an electrical power of 300 W, corresponding to an average plasma power density of 2.2 W/l, and with a duration of 15 s, and

4) <u>amine top coating</u>: exposure to an acrylonitrile/H$_2$-plasma at a pressure 0.025 mbar, said top-coating plasma being provided by an argon flow of 25 sccm, an acrylonitrile flow of 200 sccm, an AC voltage of 1000 V of 50 cycles per second, an electrical power of 430 W, corresponding to an average plasma power density of 3.2 W/l, with a duration of 60 s.

The flow values given above are set-point values in "standard cubic centimetres per minute (sccm) for the mass flow controllers used which flow controllers were

calibrated against argon flow standards except for the hydrogen flow controller which was calibrated against hydrogen.

Placing a droplet of demineralised water on a substrate coated as described above and observing the contact angle with water tested the affinity of the coating towards water. Typical contact angles were about 60 degrees.

The de-wetting behaviour was evaluated by tilting the coated substrate and observing the movement of the droplet. For typically coated substrates, a droplet rolled off the substrate leaving the surface apparently dry.

The chemical structure of the coating was analysed by infrared spectroscopy of the coated NaCl-calibration crystal.

The following absorption peaks were observed: 3369 cm^{-1} (primary amine), 2800-2900 cm^{-1} ($-CH_2-$, $-CH_3$), 2209 cm^{-1} (nitrile), 1624 cm^{-1} (primary amine), 1439 cm^{-1} ($-CH_2-$), 1375 cm^{-1} ($-CH_3$), 1060 cm^{-1} (aliphatic amine).

In infrared spectra of typical aliphatic nitrile compounds, the peak near 2210 cm^{-1} is a dominating peak. Consequently, it is a reliable indicator of the relative content of nitrile in a given sample.

In infrared spectra of typical aliphatic primary amines on the other hand, the characteristic absorptions near 3369 cm^{-1} and 1624 cm^{-1}, respectively, tend to be modest compared to the peaks arising from $-CH_2-$ and $-CH_3$.

In the present spectrum the peaks indicative of primary amine are much larger than the nitrile peak. In the light of the above considerations this is taken as evidence that the content of primary amine in the resulting poly-
5   mer coating is substantially higher than the content of nitrile.

"DNA-coupling tests"

10   The ability of the coated slides to bind (couple) single strand oligo-DNA molecules was tested in a series of experiments using the following two oligonucleotides, SGP1 and SGP3 supplied by Hobolth DNA syntese:

15   SGP1:   5` TTT CAA CAT TAG TCG TCG GTC G – NH₂  3`

     SGP3:   5` TTT CAA CAT TAG TCG TCG GTC G – OH 3`

Initially the two oligonucleotides were radio-labelled
20   with the enzyme: "T4 polynucleotide kinase" supplied by New England BioLabs. This enzyme catalyses transfer of the terminal phosphathase group of gamma P-32 labelled adenosine triphosphate to the 5`-hydroxylated terminus of an oligonucleotide. The reaction was carried out for 30
25   min at 37 °C. Labelled oligonucleotides were purified by EtOH precipitation, washed three times with cold 70 % EtOH and examined by thin layer chromatography on silica gel plates (Merck) in 0.85 M KH₂PO₄.

30   Samples of labelled oligonucleotides, and unlabelled oli-gonucleotides to be applied in hybridisation tests to a complementary oligo-sequence (see later), were prepared in four different aqueous buffer solutions with 10 pmol oligonucleotide per µl, 1) three times standard saline

buffer concentration (SSC = 0.15 M NaCl and 0.015 M sodiumcitrate at pH=7), 2) 0.50 M borate buffer of pH=10, 3) 0.150 M phosphate buffer of pH=8, and 4) a commercial buffer solution termed "ArrayIt Spot" supplied by Tele-
5   Chem International.

For each of the ten samples (SGP1- and SGP3-labelled and -unlabelled, respectively, in five different buffers), four 1 µl aliquots were spotted onto the surfaces of the
10   above-described primary amine slides for coupling of the labelled oligonucleotides with the tertiary amine coated surfaces and incubated for 4 hrs at 80 °C. Subsequently the slides were washed in water at 95 °C for 2 minutes, then washed in 95% ethanol at room temperature for 1
15   minute, and finally they were dried in a centrifuge.

The coupling of labelled oligo was monitored with an Instant Imager (Packard). The result is shown in columns A and B in Fig. 7. Spots in the rows 1, 3, 5, and 7 of
20   columns A and B are radio-labelled oligonucleotide SGP3 and SGP1 respectively, and spots in the rows 2, 4, 6, and 8 of columns A and B are unlabelled SGP3 and SGP1 re-spectively (no signal). It should be noted that each oli-gonucleotide was spotted four times next to each other in
25   the same row.

It can be seen from the figure that with the exception of SGP1 in borate buffer, clear spots have been achieved, indicating the ability of the coated surface of the
30   slides to bind oligo-DNA.

38

"DNA-hybridisation tests"

After coupling of SGP1- and SGP3-labelled tertiary amine
coated glass slides, the slides were hybridised with
5   oligo-SGP4 which can be synthesized by methods known in
the art, here supplied by Hobolth DNA syntesis, Denmark.

SGP4:   5` CGA CCG ACG ACT AAT GTT GAA A - OH 3`

10   which is complementary to SGP1 and SGP3. Prior to hybri-
disation, oligo-SGP4 was radiolabelled and purified as
described above for the oligonucleotides SGP1 and SGP3.
The probe solution for hybridisation was prepared by
adding 10 pmole/µl oligo SGP4 to an aqueous solution of 3
15   x SSC, 0.1% SDS, and 0.1% salmon sperm DNA. The solution
was then heated to 95 °C for 1 min. and subsequently
cooled with ice for 30 seconds. Prior to hybridisation
the slides were incubated for 20 min in an aqueous solu-
tion of 3.5 x SSC, 0.1 % sodiumdodecylsulphate (SDS), and
20   10 mg/ml Bovine serum albumine (BSA) at 50 °C. Subse-
quently the slides were briefly immersed in water and
then in isopropanol, and finally dried in a centrifuge.

Hybridisation was carried out over night in 100 % rela-
25   tive humidity at 50 °C. The hybridisation volume was 180
µl and the hybridisation mixture was covered with a cover
slip.

After hybridisation the slides were washed at room tempe-
30   rature in five steps:

1) The cover slip was removed by immersion in 2 x SSC,
2) 5 min wash in 0.1 x SSC,
3) 5 min wash in 0.1 x SSC,

4) 5 min wash in 0.1 x SSC,

5) drying by x min centrifugation.

Subsequently, hybridisation was monitored by a Cyclone Storage Phosphor System supplied by Packard. The result of the hybridisation is shown in the columns C and D in Fig. 8.

Significant DNA-hybridisation is seen to both oligo-nucleotides SGP1 and SGP3 for all spots.

Also, the SGP1/borate-spots, which did not show up in the coupling experiment, now clearly appear after the binding of radiolabelled complementary oligo-DNA.

In conclusion, deposition of an organic compound containing hydrogen and nitrile onto a surface through plasma treatment has resulted in an organic coating rich in primary amine. Furthermore, this coating has been demonstrated to couple biomolecules to the surface with strength and under steric conditions that allow significant interactions with other biomolecules.

EXAMPLE 2 "Primary amine plasma deposition"

3 substrates, here microscope glass slides, of length 7.62 cm (3 inch), width 2.53 cm (1 inch), and thickness 1 mm were placed in a substrate holder of an embodiment of plasma apparatus. The apparatus comprising a 30 litres cylindrical plasma chamber equipped with a three-phase electrode system shown in Figs. 3A and 3B.

The electrode geometry is illustrated in Figs. 3A and 3B showing a front view and a side view, respectively. The

electrode geometry comprises three concentric electrodes 33,34,35, an outer electrode 33, an intermediate electrode 34 circumferenced by the outer electrode, and an inner electrode 35 circumferenced by the intermediate electrode.

The outer electrode 33 is electrically shielded from the vacuum chamber walls and from the intermediate electrode by glass cylinder 32a and 32b. The outer electrode 33 consists of a 0.5 mm thick stainless steel plate bent to form a tube with a circular cross section of width 200 mm and length 200 mm.

The intermediate electrode 34 consists of a 0.5 mm thick stainless steel plate bent to form a tube with a circular cross section of width 190 mm and length 200 mm.

The outer and the inner electrodes are separated by a glass cylinder 32b of length 240 mm. The inner electrode 35 consists of a 1 mm thick stainless steel grid bent to form a tube with a circular cross section of diameter 160 mm and length 160 mm.

The electrodes were connected to a three phase 50 Hz AC-power supply with a manually tuneable voltage. The 3 substrates were evenly distributed on the substrate holder, here a tray 38, comprising a stainless steel grid which was electrically isolated from the electrodes 33,34,35 at the symmetry plane inside the inner electrode 35.

A rectangular NaCl crystal was placed together with the substrates on the tray 38 for calibration purposes.

The feed gas mixture is fed through a tube 39.

The glass slides, and the calibration crystal, were sub-
jected to two consecutive gas treatments providing a base
5   coating and a top coating, respectively:

5) <u>the base coating</u>: exposure to an Ar-plasma at a pres-
     sure of 0.07 mbar, said plasma being provided by an
     argon flow of 10 sccm, and an AC voltage 1000 V of 50
10   ` cycles per second, and with a duration of 60 s, and

6) <u>the top coating</u>: exposure to a pyrrole/H_2-plasma at a
     pressure 0.1 mbar, said top-coating plasma being
     provided by a pyrrole flow of 5 sccm, a H_2-flow of 5
15   sccm, and an AC voltage of 1000 V of 50 cycles per
     second, and with a duration of 120 s.

The flow values given above are set-point values in
"standard cubic centimetres" (sccm) per minute for the
20   mass flow controllers used which flow controllers were
all calibrated against argon flow standards.

Placing a droplet of demineralised water on a substrate
coated as described above and observing the contact angle
25   with water tested the affinity of the coating towards
water. Typical contact angles were about 30 degrees.

The de-wetting behaviour was evaluated by tilting the
coated substrate and observing the movement of the drop-
30   let. For typically coated substrates, a droplet rolled
off the substrate leaving the surface apparently dry.

The chemical structure of the coating was analysed by infrared spectroscopy of the coated NaCl-calibration crystal.

5   The following absorption peaks were observed: 3350 cm^{-1} (primary amine), 2800-2900 cm^{-1} (-CH$_2$-, -CH$_3$), 2207 cm^{-1} (nitrile), 1626 cm^{-1} (primary amine), 1446 cm^{-1} (-CH$_2$-), and 1380 cm^{-1} (-CH$_3$).

10   The peaks indicative of primary amine were much larger than the nitrile peak, indicating that the content of primary amine in the resulting polymer coating was substantially higher than the content of nitrile.

15

EXAMPLE 3 "Carboxylic acid plasma deposition"

Substrates, here 3 microscope glass slides, of length 7.62 cm (3 inch), width 2.53 cm (1 inch), and thickness
20   1 mm were placed in a plasma apparatus as described in example 2 above.

A rectangular NaCl crystal was placed together with the substrates on the substrate holder, here a tray 38, for
25   calibration purposes.

The feed gas mixture was fed through a tube 39.

The glass slides, and the calibration crystal, were
30   subjected to two consecutive gas treatments providing a base coating and a top coating, respectively:

1) the base coating: exposure to an Ar/H$_2$-plasma at a pressure of 0.1 mbar, said plasma being provided by an

argon flow of 5 sccm, a H_2-flow of 5 sccm, and an AC
voltage of 1000 V of 50 cycles per second, and had a
duration of 60 s, and

5   2) the top coating: exposure to a beta-butyrolactone/H_2-
plasma at a pressure 0.24 mbar, said top-coating
plasma being provided by a beta-butyrolactone flow of
20 sccm, a H_2-flow of 5 sccm, and an AC voltage 700 V
of 50 cycles per second, and had duration 300 s.

10

The flow values given above are set-point values in
"standard cubic centimetres" (sccm) per minute for the
mass flow controllers used which flow controllers were
all calibrated against argon flow standards.

15

Placing a droplet of demineralised water on a substrate
coated as described above and observing the contact angle
with water tested the affinity of the coating towards
water. Typical contact angles were about 30 degrees.

20

The de-wetting behaviour was evaluated by tilting the
coated substrate and observing the movement of the drop-
let. For typically coated substrates, a droplet rolled
off the substrate leaving the surface apparently dry.

25

The chemical structure of the coating was analysed by in-
frared spectroscopy of the coated NaCl calibration crys-
tal.

30   The following absorption peaks were observed: broad
absorption around 3400 cm^{-1} (O-H), 2800-2900 cm^{-1} (-CH_2-, -
CH_3), 1699 cm^{-1} (carbonyl, possibly alpha- or beta-conju-
gated carboxylic acid), 1630 cm^{-1} (C=C in conjugation with

44

carbonyl), 1448 cm^{-1} (-CH$_2$-), and 1374 cm^{-1} (-CH$_3$), 1197 cm^{-1} (C-O or O-H).

In conclusion, the base and top coatings had produced a partially unsaturated hydrocarbon coating with a high content of carboxylic acid on the calibration crystal.

EXAMPLE 4 "Thiol plasma deposition"

3 substrates, here microscope glass slides, of length 7.62 cm (3 inch), width 2.53 cm (1 inch), and thickness 1 mm were placed in a plasma apparatus as described in example 2 above.

A rectangular NaCl crystal was placed together with the substrates on the substrate holder, here a tray 38, for calibration purposes.

The feed gas mixture was fed through a tube 39.

The glass slides, and the calibration crystal, were subjected to a gas treatment providing a top coating:

the top coating: exposure to a 2,5-dimethylthiophene /H$_2$-plasma at a pressure 0.150 mbar, said top-coating plasma being provided by a 2,5-dimethylthiophene flow of 10 sccm, a H$_2$-flow of 5 sccm, and an AC voltage 1200 V of 50 cycles per second, and had duration 300 s.

The flow values given above are set-point values in "standard cubic centimetres" per minute (sccm) for the mass flow controllers used which flow controllers were all calibrated against argon flow standards.

Placing a droplet of demineralised water on a substrate coated as described above and observing the contact angle with water tested the affinity of the coating towards water. Typical contact angles were about 90 degrees.

5

The de-wetting behaviour was evaluated by tilting the coated substrate and observing the movement of the droplet. For typically coated substrates, a droplet rolled off the substrate leaving the surface apparently dry.

10

The chemical structure of the coating was analysed by infrared spectroscopy of the coated NaCl calibration crystal.

15   The following absorption peaks were observed: broad and weak absorption around 3400 cm-1 (O-H) possibly due to moisture contamination, 2800-2900 cm^{-1} (-CH$_2$-, -CH$_3$), weak 2550 cm^{-1} (S-H), 1665 and 1609 cm^{-1} (C=C and substituted C=C), 1445 cm^{-1} (-CH$_2$-), and 1375 cm^{-1} (-CH$_3$), 1226 cm^{-1}
20   (possibly C=S).

According to literature "The S-H stretching band is characteristically weak and may go undetected in spectra of dilute solutions or thin films" (see R. M. Silverstein,
25   G. C. Bassler, T. C. Morril, Spectrometric identification of organic compounds, 4'th ed., John Wiley & Sons, New York, 1980). Thus it is difficult to estimate the concentration of thiol in the produced coating.

30   However, it can be concluded, that a partially unsaturated hydrocarbon coating containing thiol groups was produced.

CLAIMS

1. A method of plasma deposition of a chemically reactive group (Y-Z) on a substrate (M), the method comprising:

(a) providing at least one precursor for the chemically reactive group, said precursor (A-X(Y)) comprising:

at least one carrier group (A), and

at least one cleaving group (X(Y)), said at least one cleaving group comprising at least one active group (Y), said at least one active group comprising one or more atoms, said at least one active group having at least one bond, at least one of said at least one bond being cleavable, and said at least one active atom being bonded to said at least one carrier group;

(b) providing at least one donor (D(Z)), said at least one donor comprising at least one addition group (Z), said at least one addition group having at least one bond, said at least one bond of said at least one addition group being cleavable, said at least one addition group being able to bond to said at least one active group,

optionally said at least one addition group (Z) being comprised in said precursor (A-X(Y)) and optionally said at least one donor (D(Z)) not being provided;

(c) providing a substrate (M);

(d) providing a gas plasma, said gas plasma having a
5   pressure and an energy to cleave said at least one
cleavable bond of said at least one cleaving group, to
cleave said at least one cleavable bond of said at least
one addition group, and to form at least one bond between
said at least one active group of said at least one
10   cleaving group and said at least one addition group, and
forming at least one activated carrier group (B); and

(e) reacting said substrate (M), said at least one pre-
cursor (A-X(Y)), said at least one donor (D(Z)) in said
15   gas plasma;

wherein the extent of said reaction of said substrate,
said at least one precursor, said at least one addition
group, said pressure of said gas plasma, and said energy
20   of said gas plasma is selected so that said chemically
reactive group (Y-Z) is bound to said substrate, either
directly (M-Y-Z) or via said at least one activated
carrier group (M-B-Y-Z), and so that when exposed to a
substance which chemically reacts with said chemically
25   reactive group, said substance binds thereto.

2. The method according to claim 1 wherein said precursor
comprises a leaving group (L), said leaving group bonding
to said cleaving group (A-X(Y)-L).

30

3. The method according to claim 1 or 2 wherein said pre-
cursor, preferably the carrier group (A), carries groups
comprising said addition group (Z).

4. A method according to any one of claims 1 to 3 wherein said active group (Y) is selected from the group consisting of oxygen, nitrogen, sulphur, and carbonyl.

5. A method according to any one of claims 1 to 4 wherein said precursor forms a primary amine $-NH_2$ on the substrate, said precursor being selected from the group consisting of: secondary amines, tertiary amines, nitriles, and combinations thereof.

6. The method according to claim 5 wherein said secondary amines are selected from the group consisting of: saturated secondary amines, unsaturated secondary amines, saturated heterocyclic secondary amines, saturated substituted heterocyclic secondary amines, unsaturated heterocyclic secondary amines and unsaturated substituted heterocyclic secondary amines, and combinations thereof.

7. The method according to claim 5 wherein said secondary amines are selected from the group consisting of: diethylamine; di-allylamine and N-allylmethylamine; pyrrolidine and piperidine; 3-methylpyrrole; pyrrole, pyrazole, and imidazole; and 2,4-di-methylpyrrole, and combinations thereof.

8. The method according to claim 5 wherein said tertiary amines are selected from the group consisting of: saturated tertiary amines, unsaturated tertiary amines, saturated heterocyclic tertiary amines, saturated substituted heterocyclic tertiary amines, unsaturated heterocyclic tertiary amines, unsaturated substituted heterocyclic tertiary amines, and combinations thereof.

9. The method according to claim 8 wherein said tertiary amines are selected from the group consisting of: triethylamine, tri-allylamine, 1-methylpyrrolidine, pyridine and pyrimidine, 1-methylpyrrole, N-vinylimidazole, and N-
5   methylpyrrole.

10. A method according to any one of claims 1 to 4 wherein said precursor forms a primary amine $-NH_2$ on the substrate, said precursor being selected from the group
10   consisting of: nitriles, saturated nitriles, unsaturated nitriles, and aromatic nitriles.

11. The method according to claim 10 wherein nitriles are selected from the group consisting of: acetonitrile, n-
15   butanenitrile, acrylonitrile, and benzonitrile.

12. A method according to any one of claims 1 to 4 wherein said precursor forms a thiol $-SH$, said precursor being selected from thiolethers, unsaturated thiolethers, sa-
20   turated heterocyclic thiolethers, unsaturated heterocyclic thiolethers, and unsaturated substituted heterocyclic thiolethers, and combinations thereof.

13. The method according to claim 12 wherein said pre-
25   cursor is selected from tert-butylsulfide; allylsulfide, allylmethylsulfide; tetrahydrothiophene; thiophene; and 2-methylthiophene and 2,5-di-methylthiophene; and combinations thereof.

30   14. A method according to any one of claims 1 to 4 wherein said precursor forms a carboxylic acid $-COOH$ on a substrate, said precursor being selected from esters; unsaturated esters; cyclic esters; or a combination thereof.

15. The method according to claim 14 wherein said precursor is selected from ethyl acetate, ethyl isobutyrate, methyl isobutyrate; methylmethacrylate, ethyl propiolate, methyl propiolate; beta-butyrolactone, gamma-valerolactone; or a combination thereof.

16. A method according to any one of claims 1 to 4 wherein said precursor forms a carbonyl chloride -COCl on a substrate, said precursor being selected from chlorinated esters, chlorinated aldehydes, and chlorinated ketones.

17. A method according to any one of claims 1 to 4 wherein said precursor is a monomer.

18. A method according to any one of claims 1-17 wherein said donor is selected from H_2, H_2O, CH_4, and NH_3 and chlorine-containing substances such as tri-chlorofluoromethane $CFCl_3$, di-chlorofluoromethane $CHFCl_2$, ethylchloride C_2H_5Cl, di-chloroethane $C_2H_4Cl_2$, chloropropane C_2H_7Cl, di-chloropropane $C_2H_6Cl_2$.

19. A method according to any one of claims 1-18 wherein said substrate is in form of a plate, a rod, a tube, a fibre, a sphere, a flake, a chip, a wafer, a membrane, cantilever, or crystal.

20. A substrate with a chemically reactive group obtainable by the method as defined in claims 1-19.

21. Use of a substrate as defined in claim 12, or as prepared by a method according to claims 1-19, in a chemical reaction between a chemically reactive substance and the chemically reactive group on the substrate.

22. Use according to claim 13 wherein said chemically reactive substance is immobilised to the chemically reactive group on the substrate.

23. Use according to claim 13 or 14 wherein said immobilised chemically reactive substance is a sensor for chemically reactive compounds, preferably biological compounds, and/or environmental compounds.

24. Use according to claim 13 or 14 wherein said immobilised chemically reactive substance is a DNA sensor.

25. Use according to any one of claims 13 or 14 wherein said immobilised chemically reactive substance is used in a method for screening of DNA, or in an apparatus for screening of DNA.

26. An apparatus for plasma deposition of a chemical reactive group on a substrate, the apparatus comprising:

(a) a vacuum pump (620,621) for providing a vacuum;

(b) a vacuum section (21A,632) comprising:

   (i) a vacuum outlet (29,633) connected to said vacuum pump,

   (ii) one or more gas inlets (28,634) for supplying one or more gasses therein, and

(iii) one or more gas outlets (29,633) for discharging residual gasses therefrom;

(c) a plasma gas section (12,635), said plasma gas section being arranged in said vacuum section for accommodating a gas plasma, and said plasma gas section comprising two or more electrodes (614,615) arranged to generate a gas of plasma therein, said vacuum section and said plasma gas section are electrically insulated;

(d) a reaction section (636), said reaction section being arranged in said plasma section and being adapted to receive at least a part of said gas of plasma, and said reaction section comprising a substrate holder (27) adapted to receive a substrate (25) and to expose said received substrate to said received gas of plasma; and

(e) a power supply (622-629), said power supply being adapted to provide one or more phases of one or more alternating voltages for providing a plasma between said two or more electrodes, said alternating voltage being supplied in one or more phases equal to or less than the number of said two or more electrodes.

27. The apparatus according to claim 26 wherein said vacuum section and said plasma gas section are electrically insulated from said vacuum section by surrounding said reaction section with an electrical insulator.

28. The apparatus according to claim 26 or 27 wherein said vacuum section is accommodated in a container, said container having a container wall of an insulating material, preferably an insulating glass material.

29. An apparatus according to any one of claims 26-28 wherein said plasma gas section and said substrate holder are arranged so that said substrate to be treated is exposed to at least a part of said gas of said plasma at
5   a fixed distance from said two or more electrodes.

30. An apparatus according to any one of claims 26-29 wherein said two or more electrodes are adapted so that surface elements of said substrate to be treated are at a
10   fixed distance from said two or more electrodes.

31. An apparatus according to any one of claims 26-30 wherein said two or more electrodes are in form of a shaped or planar solid plate.
15

32. An apparatus according to any one of claims 26-31 wherein said two or more electrodes are perforated, preferably in form of a perforated solid plate, or a grid of wires.
20

33. An apparatus according to any one of claims 26-32 wherein said two or more electrodes section are concentrically arranged with cross sections selected from the group consisting of circular, ellipsoidal, rectangular,
25   and triangular cross sections, or a combination thereof.

34. An apparatus according to any one of claims 26-33 wherein said substrate holder is arranged outside said two or more electrodes.
30

35. An apparatus according to any one of claims 26-34 wherein said two or more electrodes section constitutes said substrate holder.

36. An apparatus according to any one of claims 26-35 wherein said two or more electrodes are applied different voltages, either simultaneously or sequentially.

5   37. An apparatus according to any one of claims 26-36 wherein said two or more electrodes are arranged at different distances to said substrate.

38. An apparatus according to any one of claims 26-37
10   wherein said two or more electrodes are applied different voltages, either simultaneously or sequentially.

39. An apparatus according to any one of claims 26-38 wherein said power supply is adapted to provide one or
15   more phases of one or more alternating voltages for providing a plasma between said two or more electrodes, said alternating voltage being supplied in one or more phases equal to or less than the number of said two or more electrodes.
20

40. An apparatus according to any one of claims 26-39 wherein said one ore more phases comprises one or more conventional lamps (626-629), said lamps being connected in series with said one or more electrodes for one or
25   more of said voltages.

41. An apparatus according to any one of claims 26-40 wherein said at least one power supply comprises: a direct current (DC) power supply, an alternating current
30   (AC) power supply which at sub-radio frequencies, include a mains frequency (50-60 Hz) power supply, a low frequency (LF) power supply, and an audio frequency (AF) power supply; and which at higher frequencies include a radio

frequency (RF) power supply, and a microwave frequency (MW) power supply.

42. An apparatus according to any one of claims 26-41 wherein said at least one power supply supplying said two or more electrodes and said gas section are adapted to provide a plasma power density in the range including 0.01 to 100 W/l, preferably 0.1 to 10 W/l, more preferably 0.1 to 5 W/l, most preferably 0.1 W/l to 3 W/l, and in particular about 1 W/l.
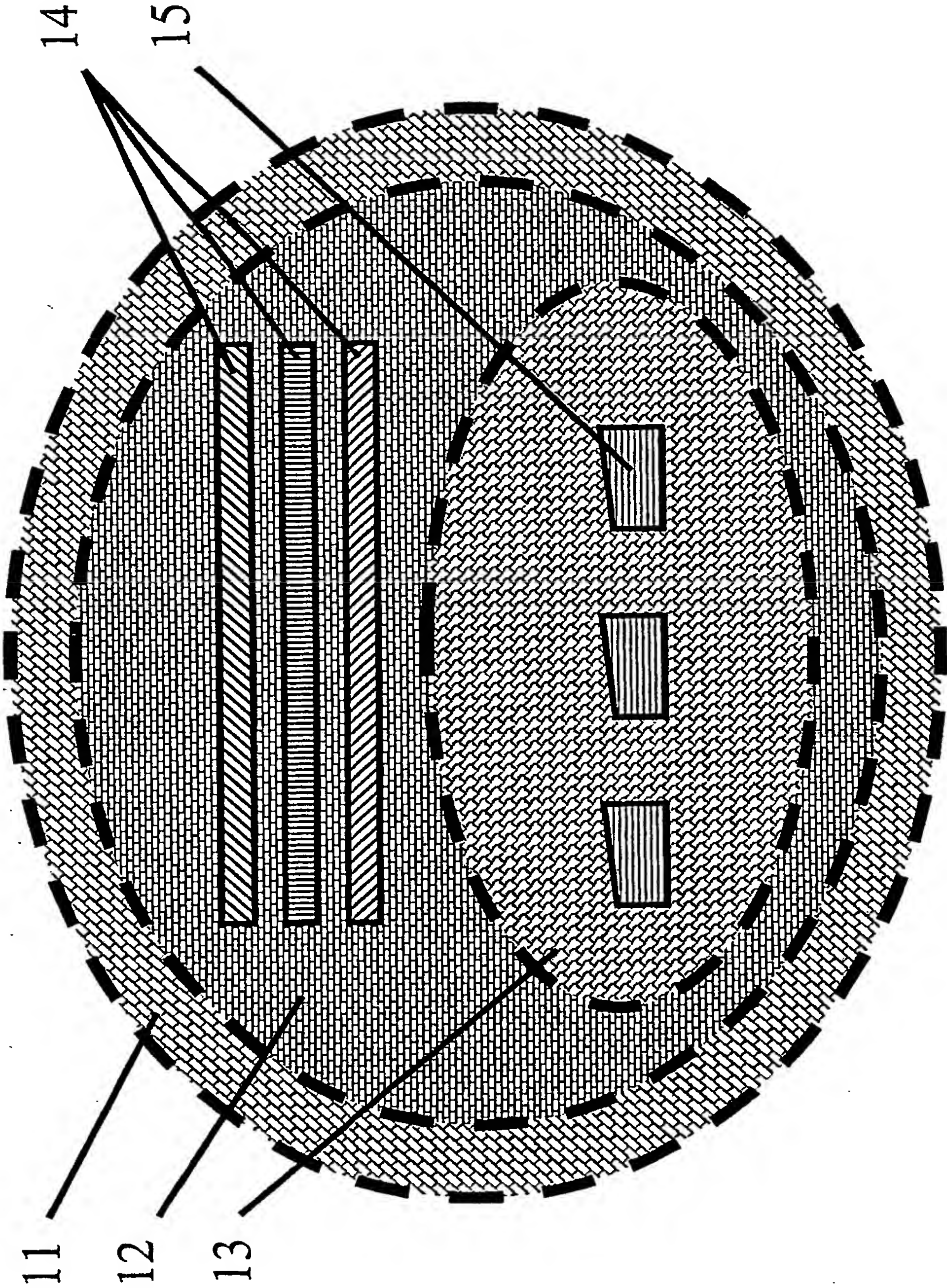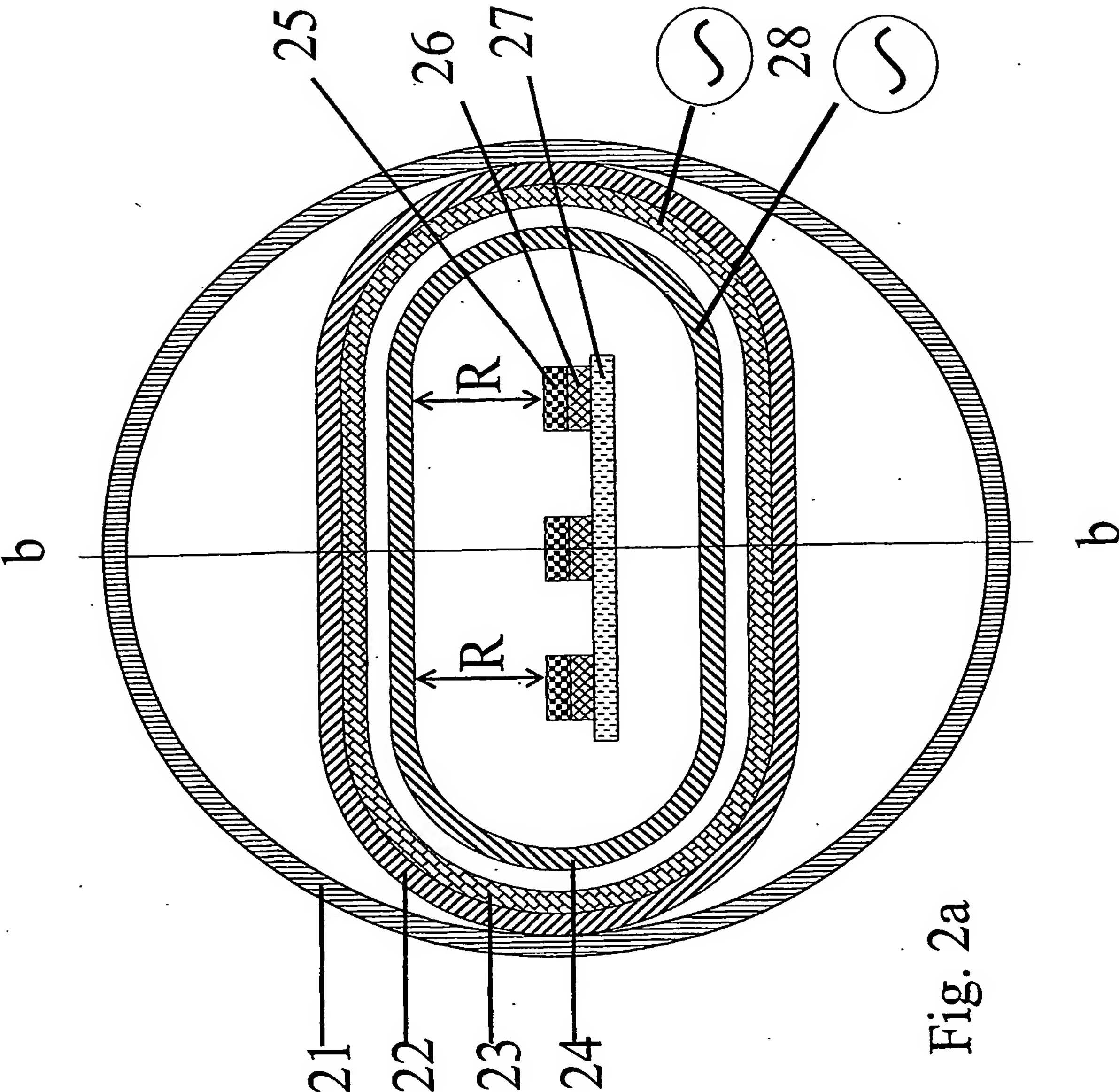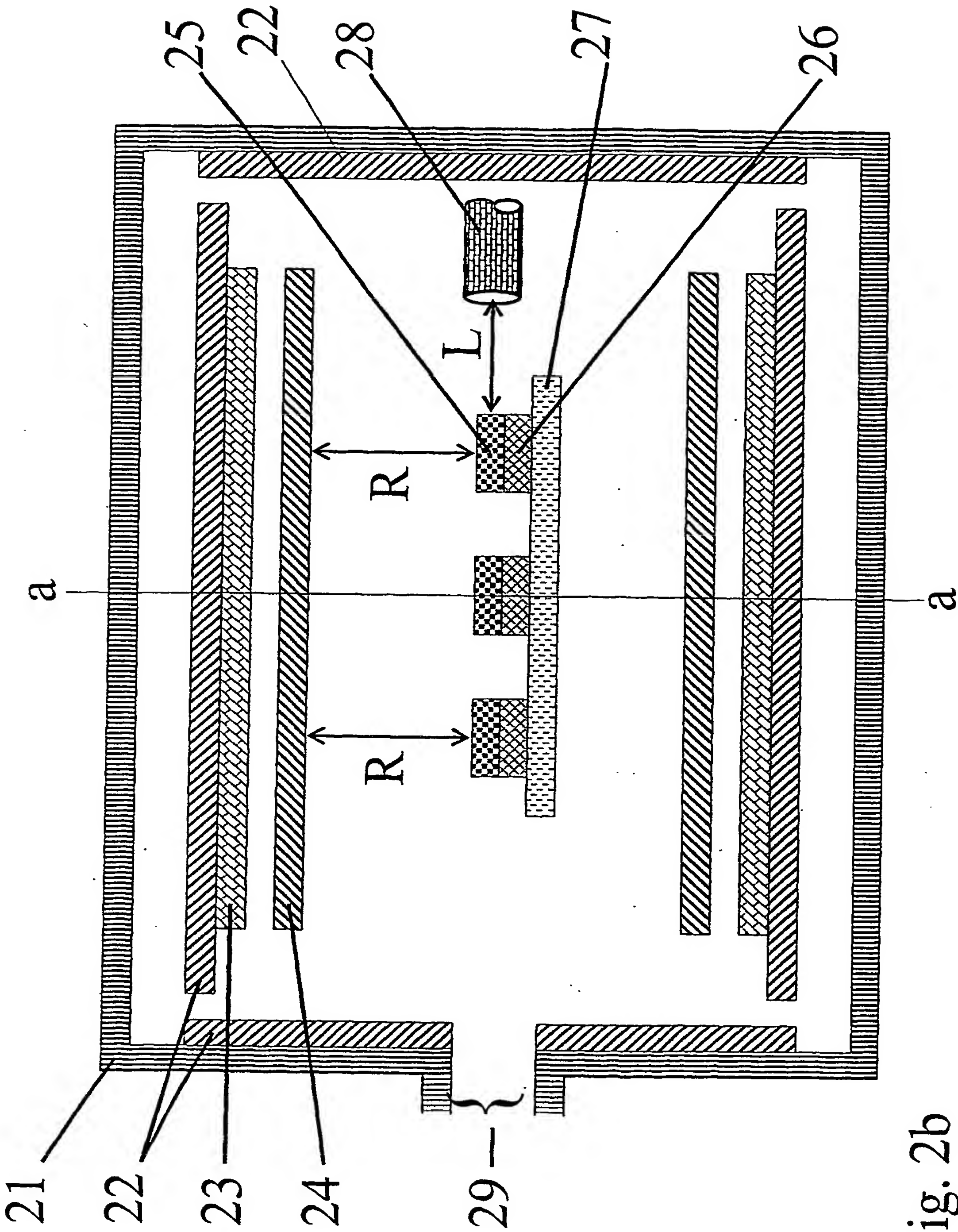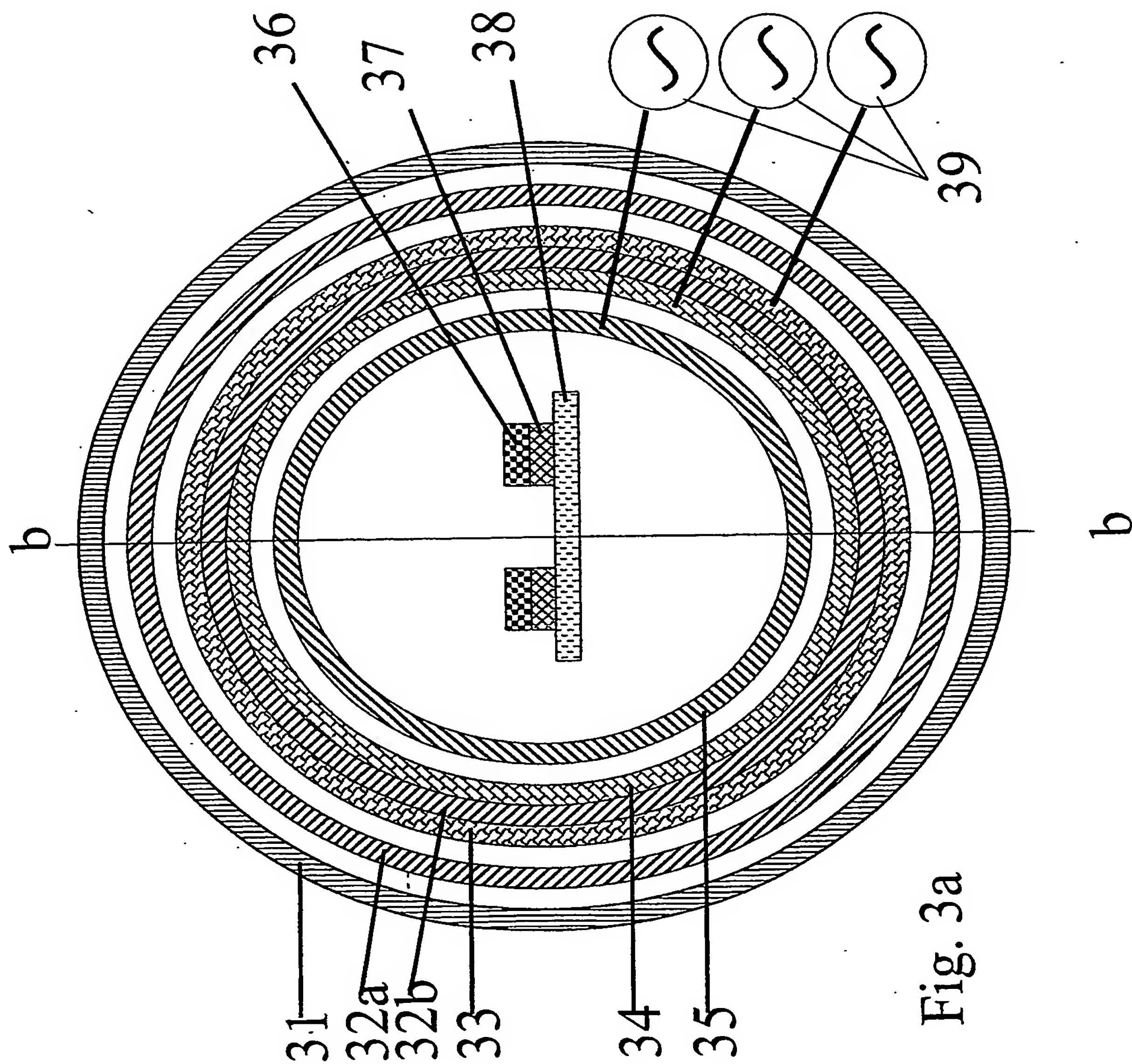
Fig. 1

Fig. 2a

Fig. 2b

Fig. 3a

Fig. 3b

Fig. 4

Fig. 5

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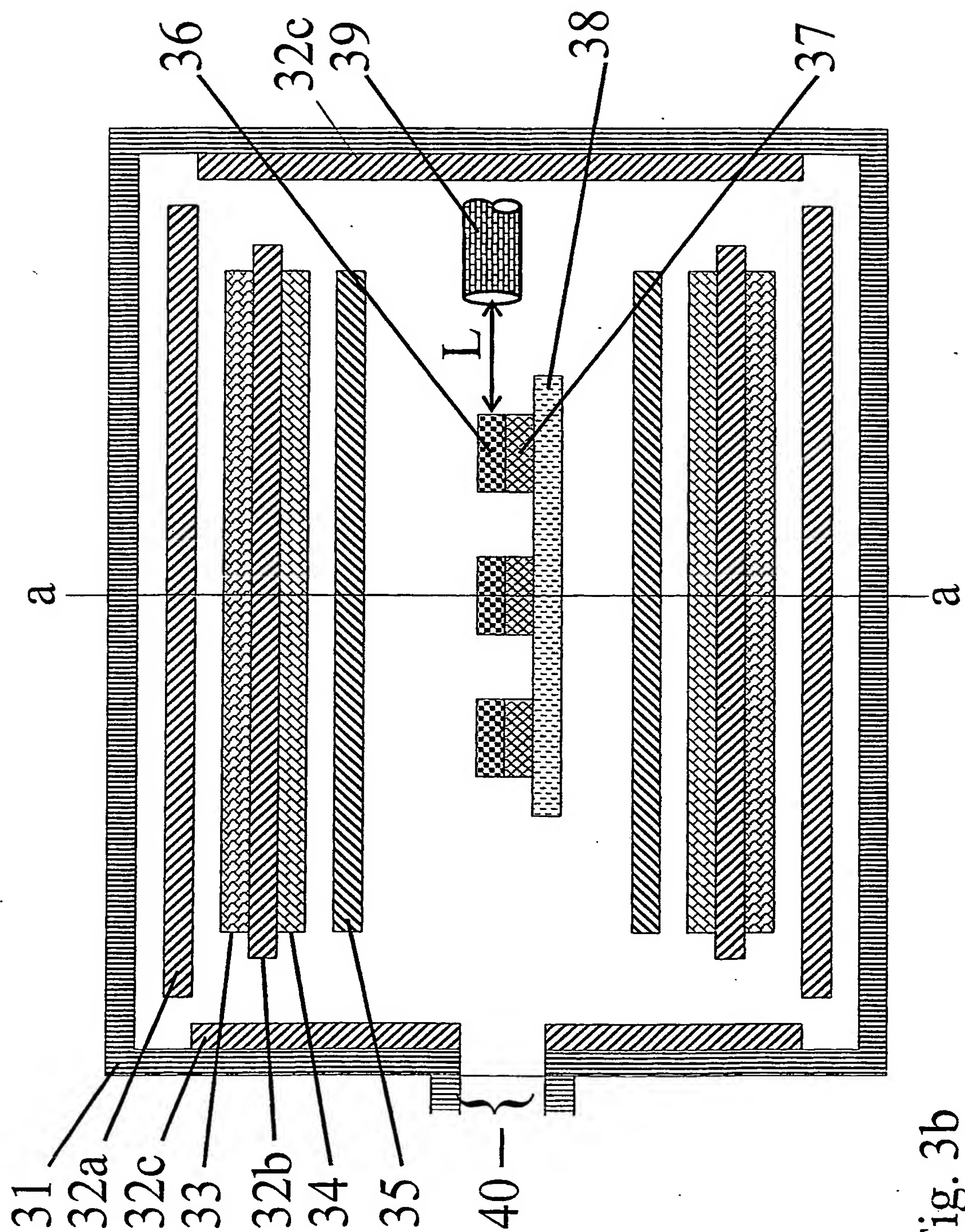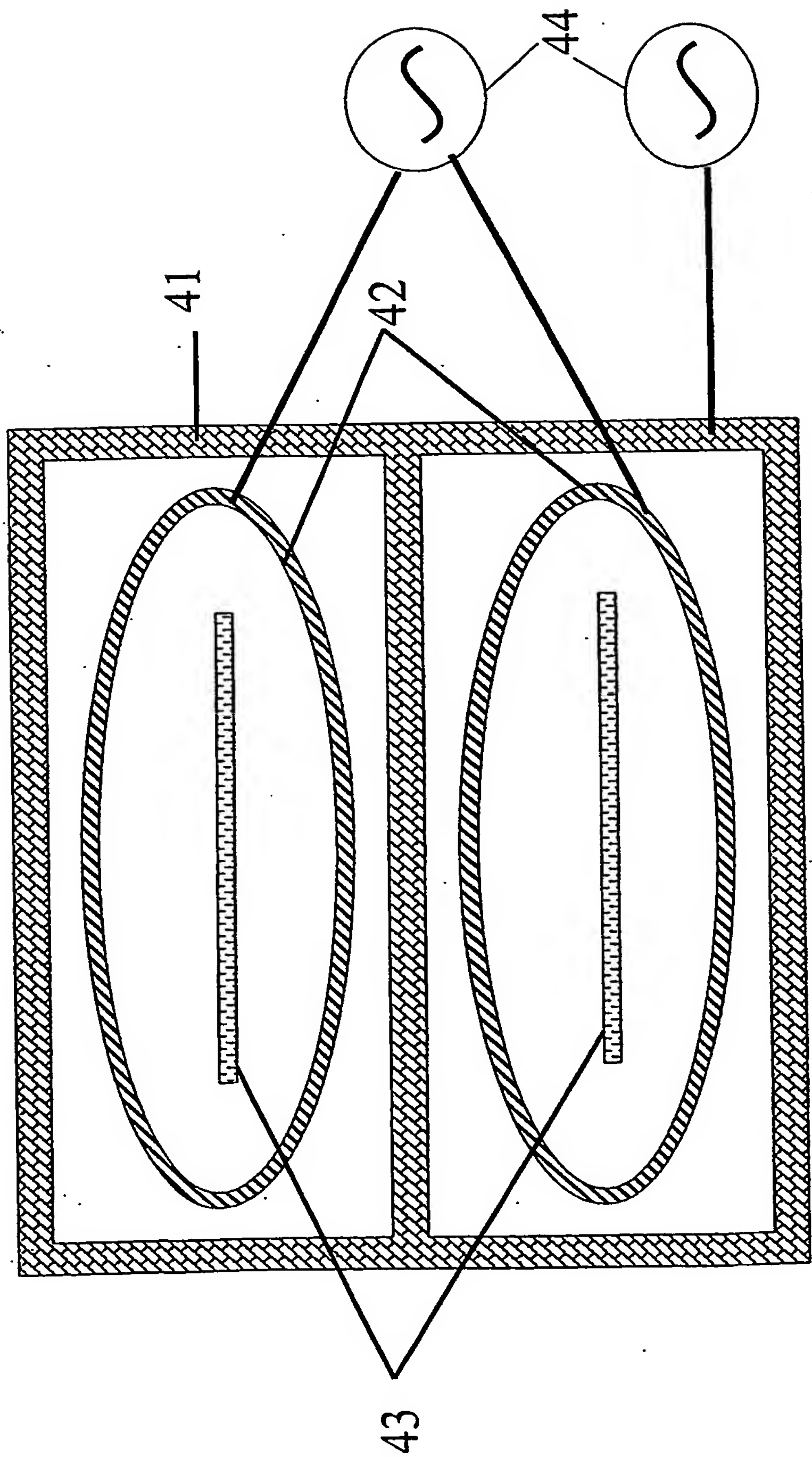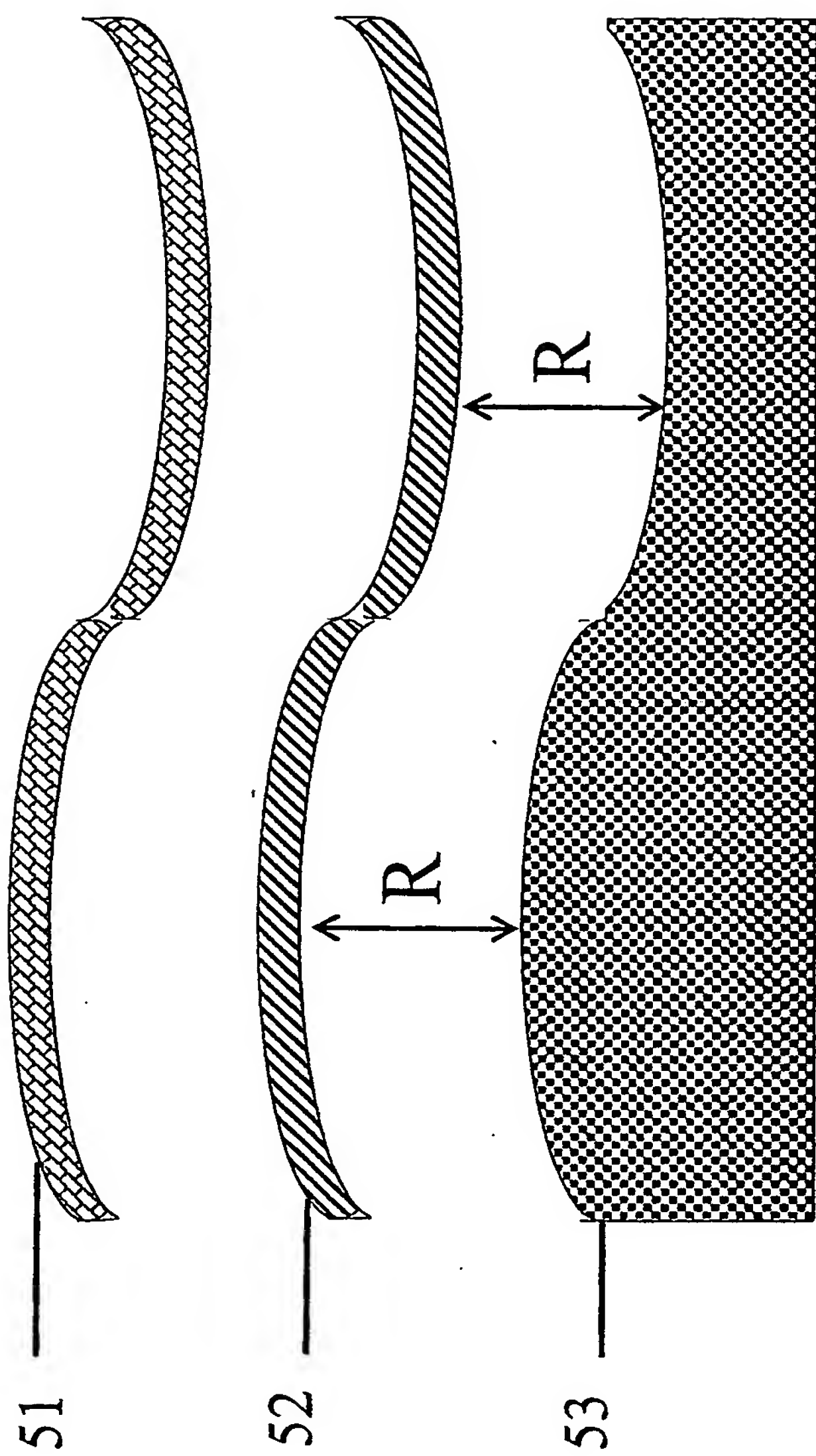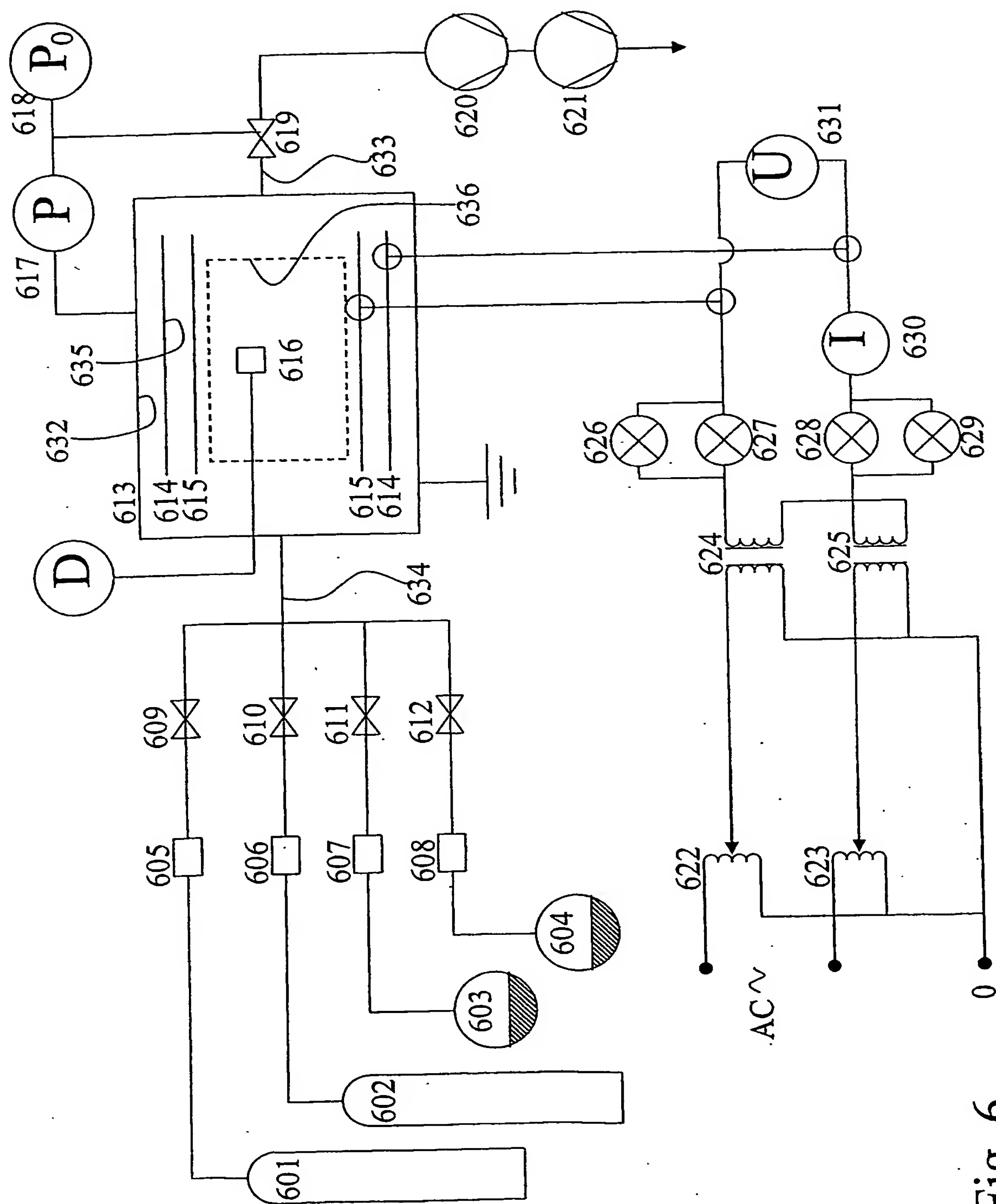
Fig. 6

Fig. 7

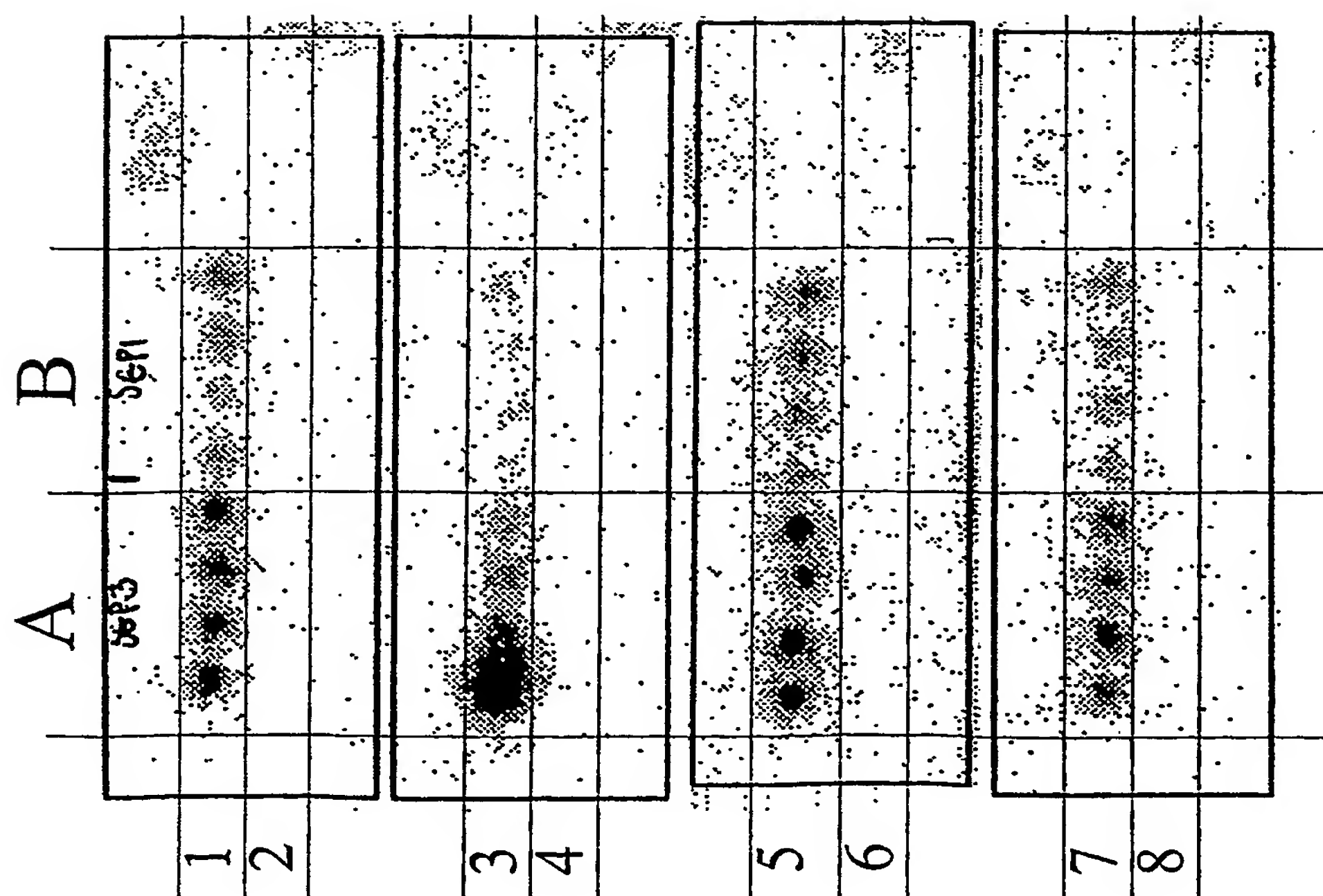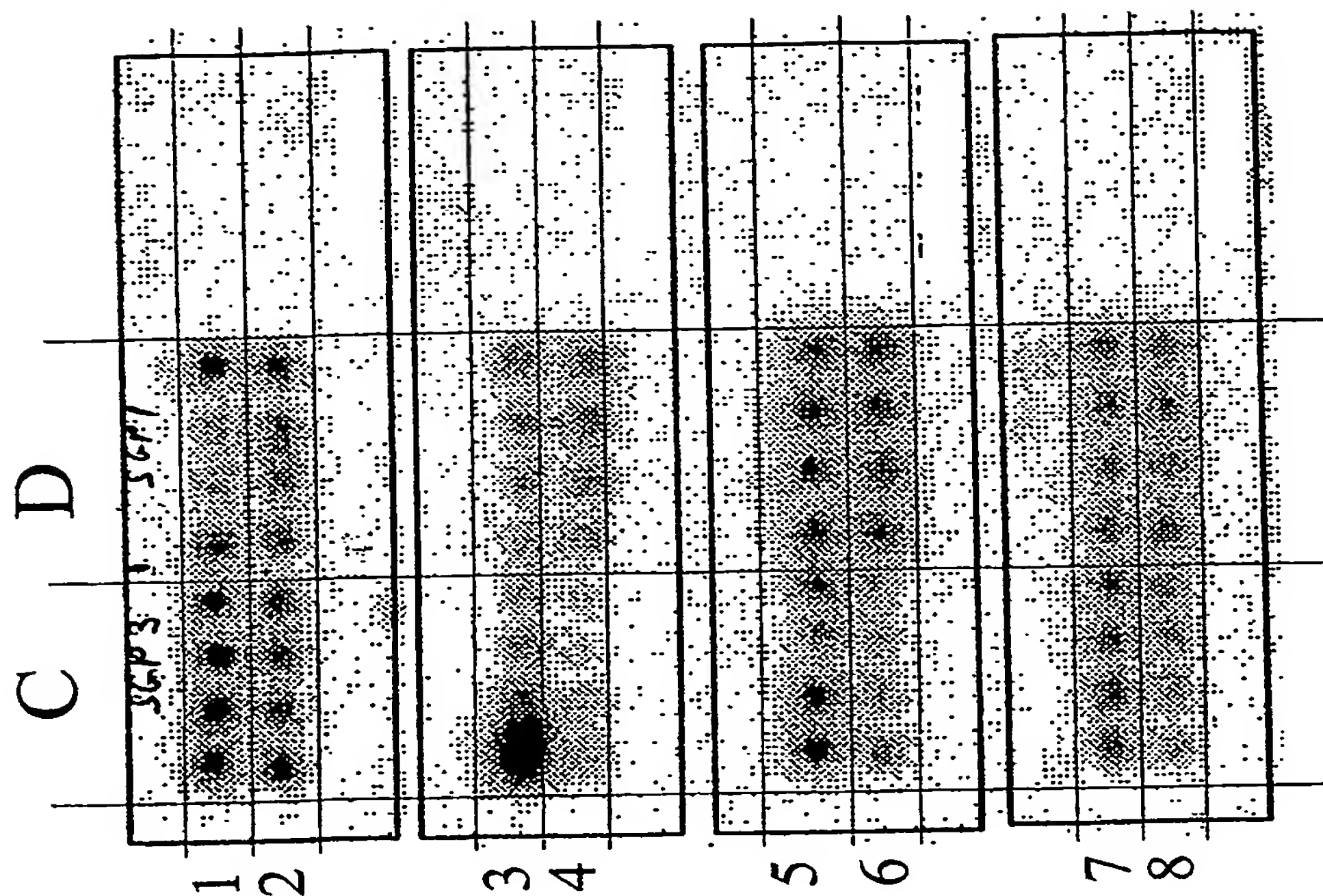Fig. 8

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   B05D7/24   A61L33/00   H05H1/24   H01J37/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   B05D   A61L   H05H   H01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 5 449 383 A (STEELE JOHN G ET AL) 12 September 1995 (1995-09-12)	
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 July 2003	21/07/2003

Name and mailing address of the ISA	Authorized officer
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